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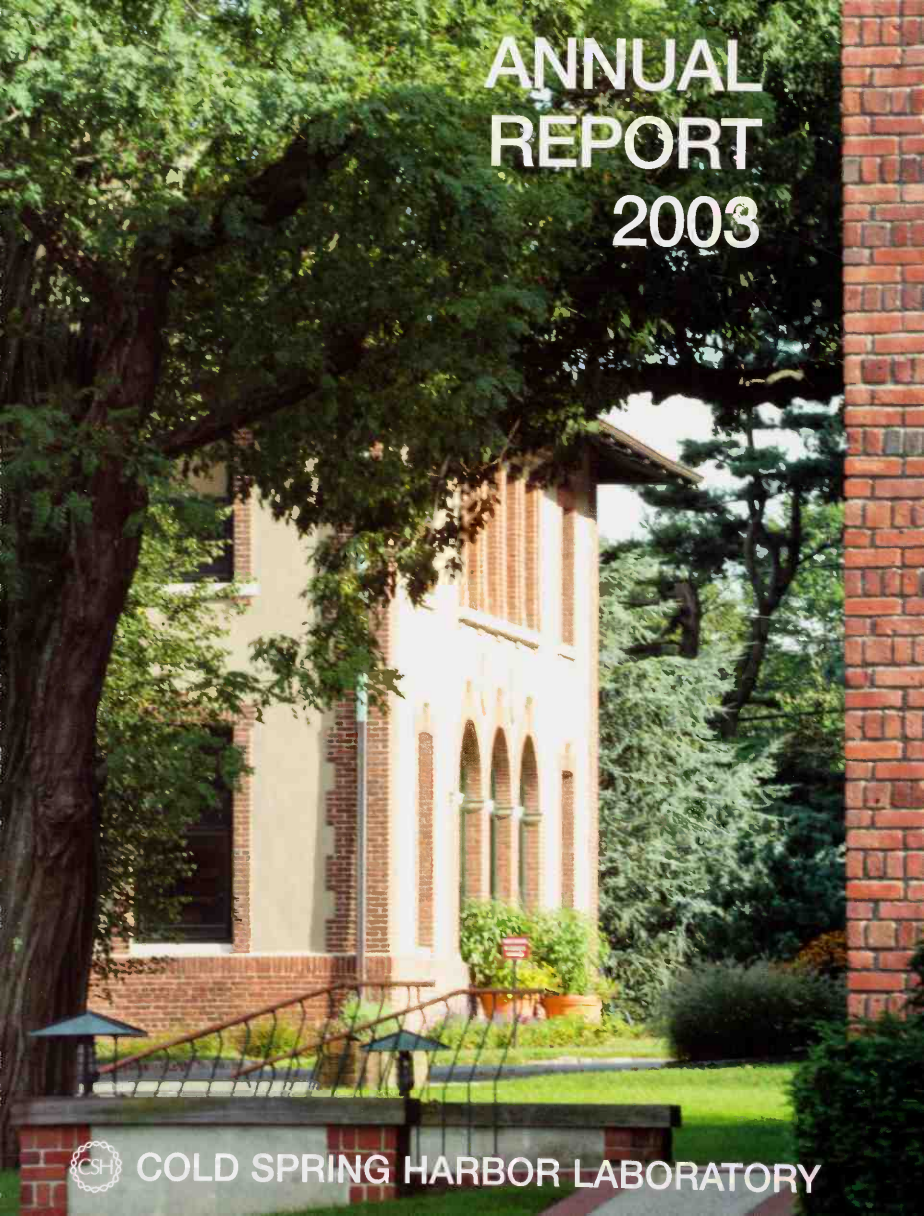

COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 2003

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ANNUAL REPORT 2003

Cold Spring Harbor Laboratory
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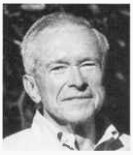

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Representation on the Board of Trustees itself is divided between business and community leaders and scientists from major educational and research institutions.

The Laboratory is chartered as an educational and research institution by the Board of Regents of the Education Department of the State of New York. It is authorized to operate a graduate program under the name "Cold Spring Harbor Laboratory, Watson School of Biological Sciences" and thereat to confer the degrees of Doctor of Philosophy (Ph.D.), Master of Science (M.S.), and Doctor of Science (Sc.D.), Honorary.

It is designated as a "public charity" under Section 501(c)(3) of the Internal Revenue Code.

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Edwin S. Marks (1926–2003)

First and foremost a family man, Edwin Marks gave to others as he did to those nearest to him. Successful in business, a generous philanthropist, provider of sound advice, and a man with broad interests and intellect, Edwin Marks died on April 24, 2003. At the time of his death, he was Vice Chairman of our Board of Trustees and a most devoted and valued advisor.

Born and raised in Brooklyn on the same block as his future wife, Nancy, Edwin's formative years occurred as his father founded and developed a successful banking business. Established in the year before Edwin was born, Carl Marks & Co. initially traded foreign securities but later, under Edwin's skilled leadership, expanded into a diverse banking enterprise including venture capital, private equities, corporate development, real estate, and financial advise and planning.

Despite—or perhaps because of—his father's success, upon entering college, Edwin did not intend to pursue a career in banking. After schooling at Brooklyn's Poly Prep Country Day School, Edwin initially studied at Princeton, but a yearning to enter into the diplomatic core caused a change in direction that greatly influenced his future. Following the advice of a family friend, he transferred to the United States Military Academy at West Point. This surprising turn of events uprooted a potentially comfortable academic life at Princeton, but due to influences from both West Point and General Maxwell Taylor, Edwin stretched himself beyond his upbringing. It was clear from discussions and e-mail correspondence with him that West Point was a special place never to leave Edwin's universe. Upon graduating, he married Nancy, and together, they set off on an adventure that lasted 54 years.

Initially stationed in El Paso, Texas and Anchorage, Alaska, environs far from the neighborhood in Brooklyn, Edwin began to use his considerable skills in an eight-year military career. His capabilities as an entrepreneur, leader, and diplomat emerged. He enjoyed serving his country.

In 1953, when his father's health began to fail, Edwin's life took what must have been an unexpected turn when he joined his father's Wall Street firm, working his way up from the bottom to learn the business and eventually head the company. Although the country lost a potential diplomat, it gained a very successful businessman who used his success and skills for the greater good. Under his leadership for more than 40 years, Carl Marks & Co. expanded and flourished. Its success enabled Edwin to achieve many other successes outside of business and pursue extraordinarily broad interests.

Edwin was not a leader who dominated, but he was a good listener and not one who stood on protocol. He was also modest and very astute. Known to answer his own telephone at work, he listened to junior colleagues and invested in their ideas. His non-business interests in music, nature, science, and medicine influenced his business as much as he used his business success to pursue these outside interests. At Cold Spring Harbor Laboratory, Edwin listened to science presentations and asked great questions of our scientists. In one case, after hearing a public lecture by Judah Folkman on anti-angiogenesis treatment for cancer, he invested in Entremed at a very early stage, an insight that eluded those of us closest to the science. Later, upon selling the overpriced stock, he was generous in making a large donation to Cold Spring Harbor Laboratory to support cancer research.

Edwin was interested in new ideas and technology. He was often one of the first to try out a new gadget or device. Once, on his way to a meeting at Cold Spring Harbor, he pulled up on the road in an electric car, one of the first on the broad commercial market. Edwin was clearly pleased when those who paused to talk to him were surprised at the absence of noise coming from the car engine. He then proceeded to impart the advantages of electric cars.

Having a great sense of humor, Edwin passed on e-mail messages with collections of jokes and anecdotes, many originating from business associates or former army colleagues. Occasionally, he would send an article or photograph that could only have come from military colleagues, but it expressed his enormous pride in the military and America.

A collector of art, music, books, and artifacts, Edwin had numerous and broad interests, many of them shared with his wife, Nancy. He loved boating, travel, and growing orchids. He was also a most generous donor to many institutions, including Cold Spring Harbor Laboratory. Edwin was Vice Chairman of Lincoln Center in New York and was particularly interested in their summer festival and *Mostly Mozart* program. He was a former Chairman of the North Shore–Long Island Jewish Research Institute and member of the Board of Directors of the Hospital system. He also supported the Right from the Start 0–3 Center at the North Shore Child & Family Guidance Center, the Smithsonian's Cooper Hewitt National Design Museum, and West Point. Together with Nancy, Edwin was a strong supporter of the Juilliard School. At Poly Prep Country Day School in Brooklyn, the Nancy and Edwin Marks Center for Science and Technology was established. Together with his sister Marjorie Boas Levins and his late brother-in-law and business partner Robert S. Boas, the Boas-Marks Medical Research Institute at North Shore–Long Island Jewish Research Institute became the focus of their clinical and basic research programs.

His long interest in medical research led Edwin to Cold Spring Harbor Laboratory in 1992 when, at the urging of then Trustee David Pall, he joined our Board of Trustees and, in 1998, became a member of the Executive Committee of the Board as Vice Chairman. The enthusiasm that Edwin had for the Laboratory and its scientists was evident by his insatiable appetite for knowing the latest research results. He understood how Cold Spring Harbor Laboratory functioned more than most and marveled in the technology and the culture. Edwin also listened to our needs and at a crucial time provided key support for the expand-

ing neuroscience research program. Upon seeing the advantages of brain imaging at the cellular level and hearing of our need to provide space for the powerful microscopes built by neuroscientist Karel Svoboda, Edwin provided the seed funds for a capital campaign to support new infrastructure. In 1999, the Nancy and Edwin Marks Building was dedicated as a research and teaching center for advanced neuroscience. The new space enabled us to begin research aimed at understanding the links between brain architecture, the molecules in the brain, and animal behavior. Edwin continued to provide support for neuroscience and cancer research. His interest enabled the Laboratory to expand while maintaining the highest quality of research.

Edwin also played a key role in the laboratory's investment and finance committees. His business experience and expert advice was offered in a calm and considered way, but had great effect. He was not afraid to ask questions but, more importantly, he knew what questions to ask.

Edwin loved to be busy, even if he was just fishing in the waters in front of Nancy and his beautiful King's Point home, listening to music or—best of all—spending time with his family. Edwin was the center of an extraordinary family that included Nancy, daughters Carolyn, Linda, and Connie, sister Marjorie Boas Levins, as well as sons-in-law and three grandchildren.

Although Edwin did not serve America as a diplomat, he served his country and community better than most. He remains an example to us all and he will be sorely missed.

Bruce Stillman
President and CEO

PRESIDENT'S REPORT

Research in biological science is advancing so rapidly that those of us fortunate enough to participate in discovery are swamped with information. As a result, we specialize, engaging deeply in one or two areas of science and relying for the rest on other specialists who condense complex ideas into short review articles in the journals. If motivated—perhaps by an unexpected discovery—we can either read further in the literature or participate in conferences and talk directly with experts in a field. Because this is how progress in science is made, the approximately 24 research meetings held each year at Cold Spring Harbor Laboratory add greatly to our intellectual environment and provide scientists working here with a constant flow of new information and fresh ideas.

The rapid pace of modern research is also making a social impact as people try to understand particular diseases or come to terms with issues that have an ethical or moral dimension, such as stem cell research. It is important for the public to keep abreast of developments in science, but it is not easy. There is broad support for the use of tax dollars to fund medical research, but many individuals want to know how research spending will help them directly. It is often a hard question to answer. Research seldom yields advances that have a direct and immediate impact on human lives. More often, practical benefits take years to develop.

Take as an example the recent outbreak of the SARS virus in 2003. Within a few months of the first reports of infection and its subsequent spread to several widely separated countries, the virus was identified and its biology understood well enough to make possible its containment. This success prevented what might have been the spread of a disease that, at best, would have wreaked economic havoc and, at worst, could have been a global medical tragedy. It is not widely enough appreciated that the ability to propagate coronaviruses and identify the genetic makeup of the infectious agent relied on basic research done in the 1960s and 1970s. And this research was not specifically geared to tracking down an emerging virus. The ability to clone and sequence the virus genome, for example, came from cancer research as much as from infectious disease investigations.

Today, vigorous advocacy for disease-targeted research drives much of the funding for basic research in biology. This phenomenon became a significant force in the 1980s, following grassroots calls for special funding initiatives to deal with AIDS and breast cancer. Although neither condition has been cured, anti-HIV drugs that emerged from an understanding of the basic biology of the virus have enabled people to live longer with chronic infection, and breast cancer mortality is dropping, mostly due to early detection.

It is almost certain that public advocacy has driven the recent increases in federal research spending. The National Institutes of Health (NIH), a collection of mostly disease-focused research institutes, supports the vast majority of basic and applied biology and medical research in the United States. The individual Institutes fund research that is done primarily in universities or research institutions such as our own. From 1999 to 2003, the NIH budget doubled to a level that is now about half the annual amount spent on defense research, not including other defense-related research on space, energy, and transportation. But increases in NIH appropriation have slowed and now are only just keeping up with inflation. Because much of its funds are already committed to multiyear grants, there will be less money for new research proposals in the next five years. Furthermore, the emerging large federal budget

deficits will greatly reduce the ability and the will of Congress to support health-related research. In such times, it is even more important for scientists to ensure that the public and Congress understand and appreciate our research enterprise.

This probable decrease in funds comes at a time when there is much more to do in medical research than even the current budgets will support. With the completion of the human genome sequence in the past year, the pace of research is faster than ever. New and completely unexpected biological phenomena are being discovered. One extraordinary example is the ability of short fragments of RNA—like DNA, a nucleic acid—to silence the expression of individual genes. The emerging understanding of "RNA interference" or RNAi, in which Cold Spring Harbor scientists are prominent, has caused us to reassess our ideas about how genes are regulated.

Eventually RNAi, like many of today's discoveries, is likely to revolutionize how medical conditions such as cancer, diabetes, and obesity are treated. But the processes required to develop drugs that are effective and safe to use take many years. With only a limited understanding of the path from the laboratory bench to the patient's bedside, the public needs a longer-term perspective on basic research and scientists have largely failed to get this across.

A particularly informative example is the history of the development of monoclonal antibodies as drugs. As part of their immune system's defense against infection, animals and humans have a population of cells (B lymphocytes) capable of producing tens of thousands of different antibodies. A single B cell secretes a single antibody that recognizes a small, specific part of a protein, and it was a long-held dream of scientists to isolate and clone one B cell and thus obtain large quantities of its specific (monoclonal) antibody. In 1975, in Cambridge, U.K., Georges Köhler and César Milstein achieved this goal with a technical innovation that produced continuously growing B cells secreting an antibody with a single specificity. Their work was immediately hailed by the press and the scientific community as revolutionary and within ten years justifiably gained Köhler and Milstein the Nobel prize. Almost as immediately, monoclonal antibodies were talked about as "magic bullets" that could cure diseases by targeting proteins located exclusively on abnormal cells such as tumors. Optimism and expectations were high, but then reality set in.

The first monoclonal antibodies were produced from mouse cells and some were approved for human therapeutic use. The first was Orthoclone OKT3, intended to prevent cells of the immune system from rejecting transplanted kidneys. But the mouse antibody, itself a protein, was found to produce an immune reaction when injected into humans, limiting the value of the therapy and possibly causing further disease. Much more work was needed, using recombinant DNA technology, to manipulate mouse antibodies and make them unrecognizable by human immune systems. It was not until 1997, more than 20 years after the initial basic research discovery, that true monoclonal antibody therapy became a reality.

A major application was in the treatment of the B-cell cancer non-Hodgkin's lymphoma (NHL), in the form of a drug known as Rituxan®. Directed against a protein on the surface of the lymphoma cells, the monoclonal antibody initiated selective destruction of the cells. Recently, the drug Zevalin®, a monoclonal antibody linked to a radioactive molecule, has been approved for the treatment of NHL. ReoPro®, also approved in 1997, was an early success in preventing platelet cell clogging of arteries after angioplasty. The number of monoclonal-antibody-based drugs is growing and includes Enbrel® and Remicade® for rheumatoid arthritis, Herceptin® for breast cancer, Erbitux® for colon cancer, and most recently, Avastin® for blocking the blood supply to tumors of the colon. One of these drugs, Erbitux® is at the center of the current insider-trading scandal that has seen a CEO imprisoned and a popular media and television personality convicted. Yet despite considerable news coverage about this drug, the public is largely ignorant of the real processes that produced it. Far removed

from the headlines and misplaced euphoria about magic bullets is a much more complex story involving the extensive search to find suitable applications for monoclonal antibodies, the difficulty of academic laboratories to develop drugs all the way to the clinic, and the reluctance of the pharmaceutical industry to pursue completely novel approaches to drug development until the academic community provides proof of principle that the approach works.

Another example of the effective but lengthy translation of research discoveries into therapeutics is the development of the remarkable drug Gleevec® for early stage chronic myeloid leukemia (CML) and rare stromal tumors of the gastrointestinal tract. This "first in its class" drug targets a protein produced as a result of a genetic abnormality in myeloid blood cell precursors in patients with CML. A translocation between two chromosomes (9 and 22) in patients with CML was first observed in 1960, but it required the development of recombinant DNA technology 13 years later to provide the tools that enabled identification the *BCR-ABL* gene that resulted from the chromosome translocation. It took a further ten years, until the mid 1980s, to show that the product of *BCR-ABL* was a tyrosine kinase, an enzyme that modifies other proteins through addition of a phosphate group. And finally, in the mid-to-late 1990s, it took the focused efforts of a single dedicated clinical scientist, Brian Druker, with access to drugs made by Novartis to demonstrate the therapeutic potential of compounds that inhibit tyrosine kinase activity. It was not until 2001 that the FDA approved the use of the inhibitor of BCR-ABL kinase activity known as Gleevec for human cancer treatment.

Gleevec's success has prompted a rush to develop other drugs based on the genetics of human cancer. But it will take time, and unfortunately sometimes a long time, for cutting-edge research to move into the clinic, despite help from the biotechnology industry in speeding the process. Shortening the time will be a challenging task requiring improved interactions between industry and academia, changes in how applied research is supported and more funds for such research, and amendments in how intellectual property from basic research is handled. This last step could involve making it mandatory for basic research patents to be licensed nonexclusively to industry, but since changes in the law would be required, it is unlikely to happen.

The completion of the Human Genome Project and other recent advances have suffused academia and industry alike with optimism about a renaissance in basic and clinical research. Almost every day the popular press announces new discoveries that may lead to new therapies. As scientists we should be careful not to over-hype the results we report. Even if it doesn't sell newspapers or suit the needs of sound-bite television, it would be more accurate if scientists, institutional public relations staff, and the media present "breakthroughs" in the clinic not as new discoveries but as the result of a long and often frustrating research process. This kind of public education about how science works must first and foremost be the responsibility of scientists themselves. If we cannot set the tone for a broader understanding of what we do, we risk losing credibility with a public that supports science but is conditioned to expect immediate results. People must be better informed about the painstaking journey of discovery, not just the results obtained and their future potential.

In this respect, Cold Spring Harbor Laboratory was particularly active in 2003. The year in which we celebrated the 50th anniversary of Jim Watson and Francis Crick's discovery of the structure of DNA was an opportunity to inform as well as rejoice. Our Dolan DNA Learning Center created the *DNA Interactive* Web site (www.dnai.org) that accompanied a five-part television series called *DNA: The Secret of Life* about the development of DNA science and its impact on society. The series had an accompanying book of the same name, written by Jim Watson and Harvard's Andrew Berry. These three projects, all inspired by Jim Watson, reached for the same goals, not simply a celebration of the double helix and other great

accomplishments in biology and medicine, but an imaginative and powerful demonstration of science as a human activity that raises vital ethical, legal, and social issues.

The Dolan DNA Learning Center (www.dnalc.org), initially established in 1986 to teach modern biology to nearby high school students, now has a global presence through its Internet-based education programs. Its current suite of Web sites, and those that are planned, permit students and their families to learn about research and its results. *DNA From The Beginning* outlines in simple terms the history of genetics from Mendel to the Human Genome Project (www.dnaftb.org). It profiles scientists who did important work, but more importantly, it describes how they made their contributions. The *Your Genes, Your Health* Web site (www.ygyh.org) is a striking collaboration between those affected by diseases, scientists, clinicians, and the Learning Center's educators. Other Web sites deal with the origins of humans and with the thorny history of science gone awry during the 20th century eugenics movement. These individual sites complement each other and share material, and several are in development, including one on cancer. Perhaps there should be a site describing the time line for developing drugs from basic research to the bedside. Focusing on approved and successful drugs and working back to the ideas and techniques that lead to treatment, we would learn valuable lessons about how basic research and medicine intertwine and about the time lines that are necessary to deal with complicated human diseases.

The type and level of biology and medicine now being taught in the middle grades of high school with the assistance of the Dolan DNA Learning Center's Web sites are exactly what the public at large must understand to participate effectively in national dialogs about disease research and issues such as human stem cell use. These sites are supported through numerous short-term grants because there is no endowment to support the Learning Center. It is a priority to seek such support. The Learning Center is one of several means by which Cold Spring Harbor Laboratory is pioneering science education, and whether or not you are a scientist, browsing the virtual world of biology and medicine may be well worth your while.

Education about the research behind existing drugs will aid in public education, but it will not advance current research. A recent *Fortune* magazine cover story by Cliffton Leaf argues that current research is not winning the fight. The claim is that academics are focusing on basic research and not applied science. Leaf also argues that there is a lack of focus on the main problems such as metastasis and early detection. The academic community, however, is very well aware of problems that need to be studied, and in this respect, the article presented little new information. For example, it has been well known that early detection of cancer increases the effectiveness of current therapy and because of this, the National Cancer Institute (NCI) leadership initiated a large program on early detection in the mid-1990s, a point not made in the article. I am now on an NCI committee to advise on the discovery and application of new technologies for cancer early detection, diagnosis, and therapy, and new approaches will emerge from such deliberations.

We know what to do—the problem is how best to achieve results. Current NIH funding mechanisms limit the amount of support each scientist can receive, and such funds cannot be pooled to make the research enterprise more efficient or goal orientated. Each pot of funding is restricted to the aims of individual grants. Funding mechanisms are needed that allow teams of our scientists to join together and work with sufficient financial resources to achieve defined goals. Without new NIH funding mechanisms, we must solely rely on long-term and significant philanthropic support. With such support, scientists at Cold Spring Harbor Laboratory can make a real difference.

HIGHLIGHTS OF THE YEAR

Research

Cancer

Scott Lowe has made an experimental breakthrough in "combination therapy" or the simultaneous use of two or more anticancer agents for treating the disease. Precancerous cells can be eliminated from the body by a self-destruct mechanism called programmed cell death or apoptosis. A hallmark of most cancers is a defect in apoptosis that enables precancerous cells to survive and proliferate. Because many traditional chemotherapy agents act in part by triggering apoptosis, such agents are frequently ineffective against tumors with defects in apoptosis.

Scott Lowe

Scott and his colleagues reasoned that using one drug to restore apoptosis (e.g., the mTOR inhibitor rapamycin) and another drug to trigger the process (e.g., doxorubicin) might be an effective way to treat cancer. They have shown that whereas rapamycin or doxorubicin treatment alone are ineffective against a mouse model of B-cell lymphoma, a rapamycin/doxorubicin combination therapy leads to remission of the disease. The study demonstrates that treatment decisions are best guided by knowledge of which gene products are functional or nonfunctional in a particular tumor, and it has produced a new paradigm for understanding and overcoming drug resistance in cancer patients.

Mike Wigler and Scott Lowe have collaborated with David Mu and Scott Powers of the Genomics Division of Tularik, Inc. to discover a previously unknown oncogene. The gene, called *KCNK9*, is expressed at abnormally high levels in half of the breast cancer specimens examined in the study and is similarly overexpressed in a large proportion of lung cancers. The experimental overproduction of *KCNK9* promotes tumor formation in mice, suggesting that elevated *KCNK9* levels may be sufficient to trigger the development of cancer. The discovery of *KCNK9* is significant because it reveals both a previously unrecognized mechanism for oncogene action (namely, potassium channels) and an attractive target for the development of novel cancer therapies.

KCNK9 was discovered by using a method that compares two sets of DNA (e.g., normal vs. cancer cell DNA) and reveals segments of DNA that are either deleted or amplified. The method, called *r*epresentational *d*ifference *a*nalysis (RDA), was previously developed by Mike Wigler and Nikolai Lisitsyn. Recently, Mike, Rob Lucito, and their colleagues have devised a new RDA-based technology that is one of the most powerful methods that now exists for profiling the genetic basis of cancer. The method is called *r*epresentational oligonucleotide *m*icroarray *a*nalysis or ROMA. Mike and Rob have already used ROMA to uncover a striking collection of chromosomal deletions and amplifications associated with breast cancer through a collaborative study with Larry Norton of Memorial Sloan-Kettering Cancer Center and Scott Powers. Mike and Rob intend to use ROMA to survey a large number of breast, ovarian, and pancreatic tumors and cell lines, as well as leukemias and lymphomas.

Greg Hannon continues to make great strides toward developing RNA interference (RNAi) as a tool for manipulating gene expression in mammalian cells. Greg and his colleagues have demonstrated that they can use RNAi to efficiently and permanently silence the expression

Greg Hannon

of virtually any gene (or genes) in cultured cells or in living animals. These findings enable them to use RNAi to carry out in-depth studies of tumor biology and treatment response, to design high-throughput screens to discover and validate new therapeutic targets, and to pioneer the use of RNAi itself as a potential therapy. As the first step toward opening that broad pipeline of cancer therapy discovery, Greg's lab has constructed a human genome-wide library of RNAi-based silencing vectors. In addition to using the library for his own work, Greg has made—and will continue to make—the library widely available for use by researchers throughout industry and academia.

Genomics and Bioinformatics

Lincoln Stein is pioneering the development of several powerful computer-based methods for analyzing a wide variety of biological data. For example, Lincoln's group has collaborated with the USDA and Cornell University scientists to create a database called Gramene* (www.gramene.org). Rice, wheat, corn, barley, and rye provide humanity with some 90% of its calories worldwide. All of these crop species are relatively closely related grasses with similar genome structures bearing many blocks of "colinear" genes. Gramene enables researchers to efficiently compare genome sequence data from these grasses and to identify genes of interest that may be used to develop new crop varieties with improved agricultural characteristics. (*The name Gramene is based on the Latin "gramen" meaning "grass" and on the Grameen Bank, which makes loans to the rural poor in emerging economies.)

Lincoln is also a principal participant in the International HapMap Project (www.hapmap.org) which released its first results in 2003. The project involves mapping the locations of variations in the human DNA sequence, many of which may ultimately be found to underlie specific diseases or differential response to therapeutic intervention. The goal of the project is to help researchers discover genes associated with cancer, heart disease, diabetes, neurological disorders, and other ailments, as well as genes associated with treatment response.

Lincoln Stein

By applying a "methylation filtration" method they recently developed that captures gene-rich regions and excludes the vast majority of repetitive, gene-poor DNA, Dick McCombie and Rob Martienssen have achieved a dramatic shortcut to sequencing the genes of corn. The approach should provide a similar boost to the sequencing and comparative analysis of other genomes in a wide variety of biological, biomedical, and biotechnological settings.

Biochemistry

A Holy Grail of biochemistry is to reconstitute the activity of complex cellular machinery in the test tube from highly purified components. Only when such purity is achieved can researchers be sure that they have identified everything that is required—and nothing that isn't—for cells to carry out all the steps of a particular biological process. After several years

of productive effort, Nouria Hernandez and her colleagues recently achieved the first such reconstitution of human RNA polymerase III transcription.

Nouria Hernandez

RNA polymerase III uses the DNA of certain genes as a template from which it builds RNA transcripts, which in turn carry out a variety of essential cellular functions. Nouria's work has defined the entire molecular machinery required to carry out all steps of the transcription process (initiation, elongation, and termination) by human RNA polymerase III. Moreover, her studies have revealed an unexpected but intriguing role of another enzyme, called casein kinase II, in transcriptional regulation. Nouria's lab has shown that by phosphorylating different components of the transcription machinery, casein kinase II can either stimulate or inhibit transcription by RNA polymerase III. Cancer and many other diseases frequently stem from defects in transcriptional regulation. Nouria's results are therefore significant from both a basic research and a biomedical perspective.

Neuroscience

Most brain neurons are produced during embryonic development. However, several regions of the adult brain continue to spawn large numbers of neurons through the proliferation of neural stem cells. Moreover, it is becoming clear that these new neurons are integrated into existing brain circuitry. Grisha Enikolopov and his colleagues have discovered that a molecule called nitric oxide (NO) is a pivotal, natural regulator of the birth of new neurons in the adult brain. Grisha, Mike Packer, and Yuri Stasiv have shown that blocking NO production stimulates neural stem cell proliferation and hence dramatically increases the number of neurons that are generated in the brains of adult rats.

Importantly, the new neurons that arise as a consequence of blocking NO production display properties of normal neurons, and they appear to contribute directly to the architecture of the adult brain. Grisha's work suggests that modulating NO levels might be an effective strategy for replacing neurons that are lost from the brain due to stroke or neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease.

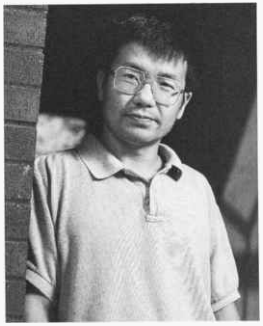
Zach Mainen

Thousands of odors that smell the same to us, or that we cannot perceive at all, are quickly recognizable as distinct and potentially meaningful odors to rodents and other animals. By studying how rodents can rapidly and accurately discriminate one odor from another, Zach Mainen is uncovering fundamental principles of brain function that apply in many settings, including how our own brains work. Zach and his colleagues use olfaction as a model system for exploring how groups of neurons participate in perception, motivation, decision-making, and ultimately consciousness.

Recently, Zach and Naoshige Uchida found that rats get a complete sense of an odor with each sniff, and they speculated that rodents probably use a rapid series of such olfactory snapshots to solve complex problems like tracking the source of an odor. Humans are far more attuned to the visual world. However, according to Zach, the neural

mechanisms that enable rodents to identify an odor in a single sniff may be similar to those that help humans take in an entire visual scene in a single glance. Part of Zach's work involves mapping the patterns of nerve cell activity in a rat's brain when the animal smells various odors. By discovering how the brain assigns identity and meaning to odors—and guides behavior based on such information—Zach and his colleagues are revealing new principles of neurobiology.

Linda Van Aelst studies the Ras and Rho family of proteins, which function—together with regulatory proteins called GAPs—as molecular switches that control a wide variety of important biological processes, including cell proliferation, cell morphogenesis, cell adhesion, and cell migration. Linda and her colleagues have recently explored the role in brain development of a particular RhoA GAP called oligophrenin-1. Loss of oligophrenin-1 function (e.g., as a result of a mutation in the corresponding gene) has been previously associated with X-linked mental retardation. In mouse RNA interference experiments, Linda has found that oligophrenin-1 is required for nerve cell structures called dendritic spines to form normally in a region of the brain required for learning and memory called the hippocampus. Moreover, Linda and her colleagues showed that the effect of decreased oligophrenin-1 on brain development can be mitigated by a drug that inhibits RhoA signaling. The latter finding may ultimately lead to treatments for X-linked mental retardation.



Yi Zhong

Humans obviously differ from the fruit fly *Drosophila* in many ways. Nevertheless, because we have much in common with it (e.g., thousands of very similar genes, a nervous system, and an ability to learn and remember), studies with fruit flies have revealed a great deal of information about human biology. Yi Zhong is now using the power of *Drosophila* research to explore the causes of Alzheimer's disease and to drive therapeutic discovery.

Versions of proteins implicated in Alzheimer's disease (e.g., amyloid precursor protein) are present in the *Drosophila* brain, suggesting that the functions disrupted in Alzheimer's are conserved in the fly. Yi and scientists at Novartis have taken advantage of this fact and have shown that expressing a pathogenic fragment of amyloid precursor protein (human Aβ42) in the *Drosophila* brain causes nerve cell death, plaque formation, and memory deficit reminiscent of Alzheimer's disease. Research using mouse models of Alzheimer's are still essential. However, Yi's work establishes a powerful alternative for testing new theories about how the disease develops and progresses, for identifying new gene or protein targets for treating Alzheimer's disease, and for rapidly screening large numbers of drugs for their therapeutic potential.

Plant Molecular Biology and Genetics

Stem cells generate new organs and tissues throughout the lives of plants—sometimes for centuries. The size, shape, location, and many other properties of such organs and tissues are determined by a complex process called stem cell patterning. One aspect of stem cell patterning involves the movement of regulatory proteins from cell to cell through channels, unique to plants, called plasmodesmata. To begin to reveal the components, architecture, and functions of plasmodesmata, Dave Jackson and his colleagues have isolated a number of mutations that affect the transport of a test protein through plasmodesmata. Consistent with the notion that transport through plasmodesmata is important for stem cell patterning, some of the mutations display developmental phenotypes. In addition, because devastating

Marja Timmermans

plant viruses frequently spread through plasmodesmata, Dave's work may ultimately reveal new strategies for controlling such viral infections.

Marja Timmermans is investigating the role of stem cell patterning in the proper outgrowth of leaves and in generating the distinct top and bottom layers of leaves. She and her colleagues have isolated the *Rolled leaf1* gene and determined that the transcription factor it encodes specifies the top layers of leaves by conveying patterning signals from stem cells. Interestingly, Marja's lab has also shown that *Rolled leaf1* gene expression is spatially regulated—by RNA interference—through the inhibitory effect of a microRNA (miRNA166) that is complementary to a site in the *Rolled leaf1* messenger RNA. Marja's observations strongly support the emerging idea that microRNAs constitute a vital and widespread class of developmental signaling molecules in plants and animals.

Cold Spring Harbor Laboratory Board of Trustees

As is customary each November, Cold Spring Harbor Laboratory welcomed new members to the Board of Trustees. David Botstein, Ph.D., director of the Lewis-Sigler Institute at Princeton University, joined our Scientific Trustees. David was a trustee at Massachusetts Institute of Technology in the 1980s. Alan C. Stephenson, James M. Stone, Edward Travaglianti, and Roy J. Zuckerberg were welcomed as individual trustees.

Ed Harlow

Lorne Mendell

Edward Harlow and Lorne Mendell concluded their terms and were honored for their outstanding service at the Board's November meeting. As noted in previous pages, we were saddened to learn of the death of our Vice Chairman, Edwin Marks, earlier in the year.

Edwin Marks

Laboratory Senior Management Restructured

After directing the Laboratory since 1994, I was named President of Cold Spring Harbor Laboratory by vote of the Board of Trustees on November 8. I will maintain my positions as the Chief Executive Officer and as Director of the CSHL Cancer Center. Through a simultaneous vote, Jim Watson was named Chancellor, after serving as Cold Spring Harbor Laboratory's President since 1994. This new position reflects his continuing role in supporting the Laboratory's educational mission through its programs for high school, undergraduate, and graduate students, especially through its Watson School of Biological Sciences, named after him at its inception in 1998.

Following these new appointments, Dr. Hollis Cline accepted the position of Director of Research. Holly is the Charles Robertson Professor at Cold Spring Harbor Laboratory and has made major contributions to the understanding of brain development and function. Holly has been serving as the Laboratory's Associate Director of Research since August 1, 2002. She will continue to serve in similar capacities, with increased involvement in the future research directions of the Laboratory.

Laboratory Employees

Long-term Service

The following employees celebrated milestone anniversaries in 2003.

35 years	James Watson
30 years	Laura Hyman
25 years	Patricia Maroney, Christopher McEvoy, Charles Schneider, Beatrice Toliver, Jeanne Wiggins, Michael Wigler
20 years	Joan Ebert, Margaret Falkowski, Winship Herr, Daniel Jusino
15 years	Margot Bennett, Francis Bowdren, Nancy Dawkins-Pisani, Maryliz Dickerson, Grigori Enikolopov, Jeffrey Goldblum, Barbara Gordon, Jennifer Gordon, Kim Gronachan, Lynn Hardin, Salvador Henriquez, Louis Jagoda, Guy Keyes, Ronnie Packer, Jane Reader, Margaret Stellabotte, Arne Stenlund, Regina Whitaker

New Staff

Alexei Koulakov joined Cold Spring Harbor Laboratory as an Assistant Professor in May. Alex came to us from the University of Utah where he was an Assistant Professor in the physics department. He received his graduate degree in physics from the University of Minnesota and was a fellow at the Sloan Center for Theoretical Neurobiology at the Salk Institute for Biological Studies.

Partha Mitra was appointed to the position of Professor in August and will strengthen our neuroscience program. Partha came from Bell Laboratories where he worked as a researcher in the theoretical physics department. He received his graduate degree in physics from Harvard University. Partha is also an adjunct associate professor at Cornell University Medical School, an adjunct faculty member at both Princeton University and the New York University Medical School, and a visiting associate at Caltech.

In December, Wolfgang Lukowitz was appointed to the position of Assistant Professor in the plant genetics department. Wolfgang came from the Carnegie Institute's Plant Biology Department at Stanford University. He received his graduate degree in genetics from the University of Tubingen in Munich, Germany.

Cordula Schulz arrived at the Laboratory as a Research Investigator in December. Cordula came from the Stanford University School of Medicine. She received her graduate degree in biology from the Maximilian-Ludwig University in Munich, Germany. We were also pleased to welcome Visiting Professors Matthew Ridley and William Benjamin this year.

Michael Townsend joined the Laboratory as Director of Information Technology in March. Mike was previously with Lehman Brothers in New York where he managed numerous functions and special projects including equity securities infrastructure, Y2K Disaster Recovery, and Fixed Income E-Commerce Projects.

Promotions

A number of our staff were promoted in 2003, including Tatsuya Hirano to Professor; Yuri Lazebnik to Professor; Josh Dubnau to Assistant Professor; Rusiko Bourtchouladze to

Adjunct Professor; Ken Chang to Research Investigator; Diane Esposito to Research Investigator; and Peter Sherwood to Director of Research Communications.

Departures

Shiv Grewal, Associate Professor; Douglas Conklin, Research Investigator; and Luca Cartegni, Senior Fellow, all departed the Laboratory in 2003.

Chief Financial Officer G. Morgan Browne retired from the Laboratory on December 31 after 19 years of service. Although he has agreed to stay active at the Laboratory, we will miss his day-to-day work on our finances. I wish to add my thanks to Morgan for his many years of dedicated and outstanding service.

Robertson Research Fund

The Robertson Research Fund has been the primary in-house support for our scientists for nearly three decades. During 2002, Robertson funds supported research in the labs of Carlos Brody, Dmitri Chklovskii, Josh Dubnau, Grisha Enikolopov, Josh Huang, Eli Hatchwell, Winship Herr, Yuri Lazebnik, Alea Mills, Vivek Mittal, Marja Timmermans, Linda Van Aelst, and Yi Zhong. It also provided support to new investigators Alexei Koulakov, Cordula Schulz, Partha Mitra, and Wolfgang Lukowitz.

Awards and Honors

On February 14, Terri Grodzicker was honored by the American Association for the Advancement of Science (AAAS) for her distinguished contributions to scientific discourse and standards as editor of *Genes & Development* (published by the Cold Spring Harbor Laboratory Press) and as Assistant Director for Academic Affairs at Cold Spring Harbor Laboratory. Since 1974, the AAAS Council has been electing those members to the rank of AAAS Fellow whose "efforts on behalf of the advancement of science or its applications are scientifically or socially distinguished."

Terri Grodzicker

DNA Interactive (www.DNAi.org), the newest addition to the Dolan DNA Learning Center Web site family, was selected as a winner of a *Scientific American* 2003 SciTech award for being one of the best scientific resources on the Web for biology. The Web site was described as "ultra-sophisticated" and the award mentioned "the folks at Cold Spring Harbor Laboratory, the brains behind the site, are to be congratulated for giving the fabric of our lives the rich treatment it deserves."

Scott Lowe, Deputy Director of the Cold Spring Harbor Laboratory Cancer Center, was awarded a $5 million, 5-year Specialized Center of Research (SCOR) grant from The Leukemia & Lymphoma Society® on October 1. With this grant, he will collaborate with researchers at the University of Minnesota, the University of California, San Francisco, and the University of Chicago to create leukemia treatments that are more effective and less toxic to patients.

On October 6, Zhejiang University in China dedicated the "James D. Watson Institute of Genome Sciences" on the Zijing Campus. It is their hope to create an "Oriental Cold Spring Harbor Laboratory" by basing it on our model as an international institute integrating scientific research and academic exchanges. It will be operated jointly between Zhejiang University

Building Dedication Ceremony in China

and the Hangzhou branch of the Beijing Genomics Institute. Several members of the Cold Spring Harbor Laboratory staff were in attendance, and Dr. Watson, who was unable to attend, delivered a videotaped speech.

DNA—The Future, a documentary directed by David Glover for Windfall Films in which Dr. Watson expresses his provocative views on DNA, science, and eugenics, was named the "Best Documentary on Science or the Natural World" by Grierson 2003. Grierson 2003 celebrates the best documentaries screened or broadcast in the U.K. between May 2002 and April 2003. The award was established in 1972, shortly after the death of the filmmaker John Grierson, and has been run by the Grierson Memorial Trust since its establishment in 1974. Grierson 2003 is an international event with awards in nine categories. This year, there were a record number of entries: a shortlist of 76 titles, and a final 36 nominations.

Dr. Roberto Malinow was selected to receive the 2003 MetLife Foundation Award for Medical Research. The award, consisting of a personal prize to the researcher and an institutional award to Cold Spring Harbor Laboratory, was established by MetLife in an attempt to treat and eventually cure Alzheimer's disease, which already affects 4 million Americans and has the potential to affect as many as 14 million by 2050. It is awarded yearly to a "smart, successful, dedicated researcher who has made significant contributions to the understanding of Alzheimer's disease" and "provides them with the means to continue their work."

Z. Josh Huang, Assistant Professor, was awarded a 2003 EJLB Foundation Scholar Research Award, totaling CAN$300,000 over three years. Up to seven outstanding young researchers are awarded each year. The EJLB Foundation was established in 1983 as a charitable corporation with an interest in medical and scientific research in all areas of neuroscience that pertain directly or indirectly to schizophrenia and mental illness.


Ira Hall


Rob Martienssen

Two papers published in *Science*, "Regulation of Heterochromatic Silencing and Histone H3 Lysine-9 Methylation by RNAi" (13 September 2002) and "Establishment and Maintenance of a Heterochromatin Domain" (27 September 2002) were recipients of the AAAS Newcomb Cleveland Prize this year. Laboratory employees Ira M. Hall, Catherine Kidner, Robert A. Martienssen, and Ken-ichi Noma, former Laboratory scientists Shiv Grewal, Gurumurthy D. Shankaranarayana, and Thomas A. Volpe, and colleagues Grace Teng, Nabieh Ayoub, and Amikam Cohen were all recognized as establishing "a new landmark in epigenetic control" through these papers. This is the oldest and largest of the AAAS awards, and it recognizes outstanding papers published in the research articles or reports section of *Science*. Each recipient receives a bronze medal and a share of the $25,000 prize.


Ken-ichi Noma

Cold Spring Harbor Laboratory was named "Institute of the Year" in the November/December issue of *Genome Technology,* which called the Laurel Hollow research and educational powerhouse "Long Island's Genomics Gem." The honor was determined based on Cold Spring

Harbor Laboratory's "recent advances in genomics," "its share of star genomics scientists" from James Watson to RNAi pioneer, Greg Hannon, *Arabidopsis* expert Rob Martienssen, and cancer genomics researcher Mike Wigler, and its broad research and educational mission. Cold Spring Harbor Laboratory Associate Professor Lincoln Stein was named "Most Innovative in Bioinformatics" in the same issue. *Genome Technology* readers—more than 20,000 scientists, researchers, technologists, and managers in advanced science research in drug discovery, molecular biology, genomics, proteomics, bioinformatics, and allied disciplining—are asked to submit nominations and votes for the "Institute of the Year" selection.

Building Projects

Several key scientific facilities were completed during the year. The Laboratory expanded its Bioinformatics center, creating much-needed new workspaces in the Williams building. The James Laboratory glassware kitchen was completely renovated and expanded to meet the growing needs of the building's scientific staff. Other scientific facilities, including a new Genome Center clean room for microarrayers and a new confocal microscopy suite in Hershey, were constructed as well. Additionally, the Laboratory's new Flow Cytometry suite was completed in the Hershey building.

The Laboratory's program to improve housing for students and scientists has made considerable progress. The first phases of an ambitious project to provide housing for 32 students at Uplands Farm have been completed. A spacious, six-bedroom residence was completed at the Uplands Field Station, and the existing Caretaker's Cottage was completely renovated. Additionally, the foundations were laid for the construction of a new structure that will provide high-quality housing for half the total residents at the farm. We anticipate that this new structure will be completed by the end of 2004. In addition to the projects under way at Uplands Farm, the Laboratory has been systematically upgrading all of its existing housing. A tiny studio apartment in the Hershey building was gutted and expanded to a spacious two-bedroom apartment, and two apartments in Hershey were renovated and modernized.

Rare red-tail hawk watching construction at Uplands Farm.

Other facilities at the Laboratory received major improvements as well. Significant emergency power and air-conditioning upgrades were made to the Network Operations Center to help cope with the dramatic increase in demand for computing resources at the Laboratory. The Laboratory's Graphic Arts facility in the Hershey building was completely renovated and expanded to help facilitate its evolution into the new Media Arts and Visualization (MAV) department. Other projects included the resurfacing of Bungtown Road and a major renovation of the Russell Fitness Center in support of the Laboratory's recreation and employee wellness programs.

Education

Meetings and Courses

The high-quality production of the 27 courses and 19 meetings hosted at Cold Spring Harbor Laboratory throughout the year was impressive as usual. Most notable this year, however, was the entire Meetings and Courses, Facilities, and Food Services staffs' profes-

Charlie Boone and Brenda Andrews on Bungtown road after the Blackout of 2003.

sional response to many unexpected and unusual challenges this year. With two very special meetings—*The Biology of DNA*, in celebration of the 50th anniversary of DNA, and *JDW: A Celebration*, in honor of Jim Watson's 35th anniversary at the Laboratory—on the agenda, the staff was prepared for a busy year without dealing with a disease epidemic, a blackout, and a major snowstorm as well.

For the first half of the year, the staff responsibly handled the dozens of questions, concerns, and fears regarding the SARS outbreak, which caused more than 8000 illnesses and more than 700 fatalities in two dozen countries, affecting the travel and safety of many of our international and national meetings and courses participants.

On August 14, most of the Northeast and Midwest were left in the dark when the United States suffered its largest blackout since 1977. For 22 hours we were without power and had 300 visiting biologists, all here for the *Yeast Cell Biology* meeting, on campus. Posters and platform sessions were rearranged, and Food Services found enough charcoal to barbecue the evening's meal. The Facilities staff worked through the night to pump fuel from the local gas station to keep the emergency generators running.

Finally, a December 5th snowstorm dumped more than a foot of snow on the area and, again, the staff ensured that disruptions to the *Molecular Approaches to Vaccine Design* meeting were minimal.

Banbury Center

The year 2003 marked the 25th anniversary of Charles Robertson's gift of his Lloyd Harbor estate of some 45 acres, the buildings on it, along with an endowment to contribute to the upkeep of the estate to Cold Spring Harbor Laboratory, creating what is now known as Banbury Center. The Silver Anniversary was celebrated with a small party on September 12. Guests included Bill Robertson and Anne Meier (two of Charles and Marie Robertson's children), former directors Victor McElheny (1978–1982) and Michael Shodell (1982–1986), and

friends from Lloyd Harbor. Harold Varmus, Nobel laureate, former Director of the National Institutes of Health and current President of Memorial Sloan-Kettering Cancer Center, was the guest of honor.

Throughout the rest of the year, there were 19 meetings at Banbury, with 654 participants. Of these, 81% came from the United States, drawn from 33 states. The participants from abroad came from 21 countries, once again showing the high esteem in which meetings at Banbury are held throughout the world. As usual, the Banbury Center program dealt with eclectic, interesting, and often controversial topics. *Taxonomy and DNA; Taxonomy, DNA, and the Bar Code of Life;* and *Integrating Progress in the Genetics and Neuropharmacology of Schizophrenia,* were of the most notable.

Dolan DNA Learning Center

In 2003, the long-awaited second edition to David Micklos and Greg Freyer's *DNA Science* was published. Now 100 pages longer, including two new lab projects and the first substantial treatment of eugenics available in a general biology text, this book is sure to continue to be largely responsible for bringing DNA experiments to advanced high school and beginning college students.

On a more global scale, *DNA Interactive (DNAi)*, the DNALC's newest addition to the World Wide Web, has already received more than 375,000 visitors and has contributed to a 23% rise in visitation to the DNALC's family of award-winning Web sites, which received 4.85 million visitors in 2003.

Clemson University (South Carolina) and the Roberson Museum of Arts and Sciences (Binghampton, New York) became the fourth and fifth licensees to use the DNALC teaching methods, Internet technology, and intellectual property, while DNALC *West*, the Singapore Ministry of Education, and the Science EpiCenter (New London, Connecticut) all fully developed their programs initiated in 2002.

In-house, the DNALC received four awards from the National Science Foundation to develop educational programs that closely parallel the work of CSHL scientists David Jackson, Dick McCombie, Marja Timmermans, and Lincoln Stein. These opportunities offer unique laboratory experiences to students and faculty alike.

CSHL Press

The CSHL Press enjoyed a very productive year. Eleven new book titles were published and a larger number of books—79,000 copies—were sold, in more countries around the world than in any previous year. Among the laboratory manuals published for working scientists, perhaps the most notable was *RNA Interference*, a guide to a new, powerful, and swiftly adopted way of silencing genes. CSHL scientist Gregory Hannon edited what became the first authoritative book on the biology and applications of this experimental approach. It has been an instant success.

Textbooks for undergraduates are a recent addition to the publishing program. James Watson's classic textbook *Molecular Biology of the Gene*—first published in 1965 and for 25 years the book of choice for advanced undergraduate teaching in genetics—was comprehensively revised and published to acclaim in December, in a fifth edition written by Richard Losick, Tania Baker, Steve Bell, Alex Gann, and Michael Levine that seems likely to restore the book to a central place in the curriculum.

In a year in which many scientific conferences, cultural events, and news reporting around the world were devoted to the 50th anniversary of Watson and Crick's proposal for the structure of DNA, the Press assembled a book to honor Jim Watson. With essays by more than 40 eminent people who have worked with Dr. Watson in all kinds of contexts, *Inspiring Science: Jim Watson and the Age of DNA,* edited by John Inglis, Joe Sambrook, and Jan Witkowski, succeeded handily in presenting a multidimensional view of its subject and his diverse and extraordinary accomplishments.

Journal publishing at the Laboratory, which began with one journal in 1987, is now a core competence of the Press. In its 16th year, the program grew to a list of five publications with the addition of the journal of The RNA Society, *RNA.* In its first year with Cold Spring Harbor Laboratory, the journal program's financial goals were achieved and its reputation enhanced by incisive peer-review and editorial decision-making and the continued publication of high-quality papers in molecular biology and genomics.

Watson School of Biological Sciences

James D. Watson raised the idea of a graduate school at Cold Spring Harbor Laboratory in the fall of 1995. Just seven and one-half years later, the Laboratory awarded its first Ph.D. degree. On May 13, 2003, Amy Caudy—of the entering class of 1999—became the first Watson School student to defend her doctoral dissertation. This fall, Ira Hall defended his thesis, after just over three years in the School.

When the Watson School established the goal of offering a four-year Ph.D. degree in the biological sciences, one concern raised was whether it would be possible for students to perform substantive research. Four years later, it has proven possible. Caudy was one of 16 international graduate students to receive the 2003 Harold M. Weintraub Graduate Student Award, sponsored by the Fred Hutchinson Cancer Research Center, based on the quality, originality, and significance of her work. During his studies, Ira Hall participated in two projects recognized in 2002 by the journal *Science* as the "Breakthrough of the Year." This year, the American Association for the Advancement of Science recognized these two studies with the 2003 Newcomb Cleveland Prize, which acknowledges an outstanding paper published in *Science.*

The 2003 student recruitment was also very successful. Of 13 offers made to applicants, 8 were accepted—more than a 60% acceptance rate. Students turned down offers from the University of California, Berkeley, Massachusetts Institute of Technology, Stanford, and others to join the School. With one deferral from 2002, there were nine entering students this year. On August 25, Hiroki Asari, Rebecca Bish, François Bolduc, Monica Dus, Angélique Girard, Christopher Harvey, Jeong-Gu Kang, Izabela Sujka, and Wei Wei began their adventure in becoming scholars in the biological sciences.

CSHL Library and Archives

In 2003, the CSHL Library continued to provide information to its scientists, both traditionally and electronically. In addition to the many new databases that the library had acquired in 2002, they tested and added several new databases and e-books—including *Current Protocols* in nine subjects—to the collection this year. InterLibrary Loans are now mostly delivered in PDF format, and WebCat, the on-line catalog, now includes most of the titles in the Library's collection, including rare books. The library has continued to participate in the BioInformation Synthesis Collaborative (BISC), which the Harvard Libraries (Museum of Comparative Zoology and Biological Laboratories Library) joined in 2003. The Archives has

undergone tremendous growth and development as well. *The Oral History Project*, which aims to document Cold Spring Harbor Laboratory history through the eyes of the scientists who worked and visited here, grew to 40 interviews and will ultimately be made available through our Web page. In conjunction with *The Oral History Project*, a second archival project was initiated this year: the *Memory Board*. The *Memory Board* is an on-line forum that paints a picture of the lab through the eyes of anyone who contributes their written recollections to the Web site. These are typically casual contributions that attempt to capture a complete view of what makes Cold Spring Harbor Laboratory special.

50th Anniversary of the Double Helix

On the morning of Saturday, February 28, 1953, Jim Watson cleared his desk in the Cavendish Laboratory at Cambridge University. His goal: To have a large, flat surface on which to explore how cardboard cutouts representing the building blocks of DNA might fit together to form the basic structure of the molecule of life. Although years of work by many scientists laid the chemical and intellectual foundations for the discovery of the double helix, the principal features of the now familiar DNA structure came together for Jim and Francis Crick over a short period in 1953.

To mark the 50th Anniversary of the double helix, Cold Spring Harbor Laboratory hosted or participated in several scientific, cultural, and celebratory events in 2003. Many of those events explored how the discovery of the double helix has transformed science, medicine, and society and rightly recognized the tremendous strides that were made in biology since that time (see pages 31–46).

Events

Symposium

The 68th Annual Cold Spring Harbor Laboratory Symposium, "The Genome of *Homo sapiens*," set a new attendance record, attracting nearly 500 scientists from around the world, and represented a remarkable bookend to half a century. Fifty years prior, at a Cold Spring Harbor Laboratory meeting in 1953, Jim Watson made the first public presentation of the double-helix model for the structure of DNA. In 1986, Jim organized a Cold Spring Harbor Laboratory meeting on "The Molecular Biology of *Homo sapiens*," during which arguments for and against a proposal to sequence the entire 3-billion-letter human genome were presented. This year, that sequence was completed.

Marking this occasion, Dr. Francis Collins, who directed the United States' later efforts to sequence the human genome, gave the 2003 Dorcas Cummings Memorial Lecture. Francis, one of the leaders of the sequencing effort, escorted the audience on a brief tour of the human genome and explained the surprises scientists found as they sequenced, namely, that the genome is much more similar between individuals than expected and the genome turned out to be much shorter than expected. CSHL Association President Trudy Calabrese welcomed the audience, where meeting participants were joined by friends from the local community, as tradition dictates. The endowed lecture is in memory of Dorcas Cummings, a longtime Laboratory friend and former Director of the Long Island Biological Association. After the lecture, scientists visited the homes of Laboratory neighbors to enjoy dinner and the chance to learn from one another.

Audience at 2003 Dorcas Cummings Memorial Lecture

Gavin Borden Visiting Fellows

On March 26, Dr. Randy Schekman, Howard Hughes Medical Institute, University of California, Berkeley/ Department of Molecular and Cell Biology, delivered the ninth annual Gavin Borden Visiting Fellows Lecture titled "How proteins are sorted in the secretory pathway." The annual Gavin Borden Lecture was started by Jim Watson in 1995 in memory of Gavin Borden, a publisher whose *Molecular Biology of the Cell* and other books made a lasting impact.

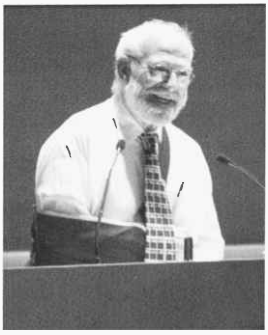
Randy Schekman

Public Lectures

This year's lectures, part of *The Double Helix 50th Anniversary Cultural Series*, focused on the practical uses of the double helix and how it has benefited humanity for the past 50 years.

April 27	David Gallo, director of special projects, Woods Hole Oceanographic Institution: *Origins—Evolution Destiny: Clues from the Deepest Sea.*
May 5	Oliver Sacks, renowned neurologist and best-selling author of *Uncle Tungsten* and *The Man Who Mistook His Wife for a Hat: Oliver Sacks: A Life of Science.*

May 12	Barry Scheck, founder, *The Innocence Project at Cardozo Law School: Barry Scheck: The DNA Defender*.
May 27	Eric Lander, one of the principal leaders of the Human Genome Project: *Eric Lander: Living in a Genomic World*.
September 21	Tim Tully, Cold Spring Harbor Laboratory neuroscientist, and Suzanne Nalbantian, C.W. Post English professor: *A Composition of Memory: From Literature to the Brain*.
October 7	Robert Shaler, director of forensic biology for the Medical Examiner's office of New York City: *Questions Answered: DNA Identification at the World Trade Center.*



Oliver Sacks

Other Lectures

The West Side School lecture series moved to a new venue: the Arthur and Joan Spiro Auditorium at the Dolan DNA Learning Center. Nouria Hernandez ("How We Read Our Genes"), Senthil Muthuswamy ("Cells Gone Wild: A 3-D Approach to Studying Cancer"), Elizabeth Thomas ("Why Two Is Better Than One: Gene Duplication in Evolution"), and Shirley Chan ("Biology on the Web") delivered these lectures, which are geared toward 4th–7th graders and their families.

As in the past, Cold Spring Harbor Laboratory hosted the Huntington Hospital Lecture Series on cardiovascular health and related diseases in both the spring and the fall in Grace Auditorium.

Concerts

Accompanying the lectures in *The Double Helix 50th Anniversary Cultural Series* was a comprehensive lineup of concerts that included an eclectic mix of musical styles from classical cello to piano duets to singers and postmodern chamber music performed by renowned young artists from around the world. They were all equally enjoyed by Meetings participants, CSHL Association members, and the general public.

April 26	Vassily Primakov, piano
May 3	Viviane Hagner, violin
May 10	Thomas Carroll, cello
May 17	Anton Belov, baritone
September 6	Ken Noda and Jennifer Frautschi, piano and violin
September 13	Antonio Pompa-Baldi and Emanuela Friscioni, piano
September 20	Tin Hat Trio, postmodern Chamber Music
October 4	Robert Belinic, guitar

Emanuela Friscioni and Antonio Pompa-Baldi

Exhibits

Matthew Schreiber, Cold Spring Harbor Laboratory's second artist-in-residence, returned on June 28 to display the holograms he created during and after his visit to the Laboratory the previous summer. The exhibit, which ran until July 16 and was open to the public, focused on the history of genetics in conjunction with the 50th anniversary of the double helix and the many important events that have taken place in Bush Lecture Hall.

After a hiatus in 2002, *FotoLab* returned to Bush Lecture Hall this year. Thirty-eight employees displayed their personal photography in *FotoLab II*. An opening reception took place on July 20, and the exhibit was open to the public until August 10.

Spend a Day with DNA

This summer, Cold Spring Harbor Laboratory teamed up with the Heckscher Museum of Art and the Cinema Arts Centre in Huntington for the first time to present *Spend a Day with DNA* on July 12, 26 and August 9, 23. Sponsored by Vytra Health Plans, the program was the first-known attempt to relate how the discovery of DNA has served as an inspiration to artists and filmmakers throughout the world.

Community Outreach

Once again, Betsy Panagot, Special Events Coordinator, organized our annual blood drive on April 14, which collected 42 pints of blood for the New York Blood Center.

Cold Spring Harbor Laboratory was well represented at the *6th Annual 1 in 9: the Long Island Breast Cancer Action Coalition 5K Walk/Run* sponsored by Cigna Healthcare on August 6. Forty-six employees participated, several of them placing, including Charles

CSHL Team at 1 in 9 Walk/Run

Kopec, Shraddha Pai, Charles Prizzi, Laurence Denis, Cara Brick, Ingrid Ehrlich, Susan Janicki, and Carla Margulies.

Institutional Advancement

Capital and Program Contributions

Private funding is critical to our research programs. Philanthropy enables the successful, innovative projects that are not yet eligible to receive public funding. For this reason, we especially appreciate our close supporters who make major gifts to our cancer and neuroscience programs. We gratefully acknowledge donors of $100,000 or more to the cancer program—the Lustgarten Foundation for Pancreatic Cancer Research, the Miracle Foundation, the Louis Morin Charitable Trust, the Dana Foundation, Manyu Ogale, and the Seraph Foundation—and donors of $100,000 or more to the neuroscience program—the Dart Foundation, Ira Hazan, the estate of William L. Matheson, the G. Harold and Leila Y. Mathers Charitable Foundation, the Simons Foundation, and the St. Giles Foundation.

Equally important to our research are the gifts with which we build and maintain our laboratories. Shortly before former Trustee William Matheson passed away in late 2002, he and his wife Marjorie announced their plans to make a gift for a future cancer building. With the gift we received this year, we are sure to build a fitting tribute to Mardi and Bill's dedication to the Laboratory's mission. Howard Solomon also gave $1 million to help fund new science buildings.

We appreciate the Starr Foundation and the anonymous donor who made gifts of $100,000 or more for the Cancer Genome Research Center. With these gifts, we will be able to continue to make advances in cancer, neuroscience, and plant genomics research at our outstanding Woodbury facility.

It is my pleasure to thank Jim Watson for his most generous donation of funds from lectures that he has presented. The resulting "2003 Fund" is an important source of unrestricted funds to further our research and education programs.

Watson School of Biological Sciences

Now in its second phase of fund-raising, led by Robert D. Lindsay, the Watson School received more than $2.4 million in gifts this year. These monies support the Dean's Chair, fellowships, and lectureships so that the Watson School can continue to grow and influence the biological sciences field. We appreciate the gifts of $100,000 or more made by the Annenberg Foundation, Curt Engelhorn, Mr. and Mrs. Alan E. Goldberg, Mr. and Mrs. Robert D. Lindsay and Family, the Ziering Family Foundation, and Joy and George Rathmann/The Rathmann Family Foundation; the gifts of $200,000 or more made by the Arnold and Mabel Beckman Foundation, the Lita Annenberg Hazen Foundation, the Florence Gould Foundation, the Miller Family Foundation, Bristol-Myers Squibb, and the William Stamps Farish Fund; and the gift in excess of $300,000 from the Charles A. Dana Foundation, Inc.

Carnegie Building

We are initiating plans to make the Carnegie Building fit for the 21st century, and for the first time in 100 years, we are looking to renovate and expand the Library and Archives. To do

so, a campaign was initiated this year to raise funds to add much-needed space and equipment to the existing structure, so that current and future generations of scientists and historians will be able to access, preserve, and study valuable archival and research materials. The new annex will include wheelchair accessibility; study space; storage, workshops, and reading areas; computer workstations; display space for the extensive archives and rare books collections; and the establishment of the Center for the History of Molecular Biology, a new academic program to document research in the 20th and 21st centuries. We are grateful to Waclaw Szybalski, Harold E. Varmus, Arnold J. Levine, Victor and Ruth McElheny, and Hiro Nawa for making the first contributions to this important campaign.

Dolan DNA Learning Center

Thanks to a generous gift from Joan and Arthur M. Spiro, the Dolan DNA Learning Center auditorium was dedicated as the Joan and Arthur M. Spiro Auditorium on September 9. Mr. Spiro is a member of the Cold Spring Harbor Laboratory Board of Trustees, an Executive Committee member, Chairman of the Dolan DNA Learning Center Committee, and Trustee Liaison for the DNALC's Corporate Advisory Board. We also thank Laurie J. Landeau, V.M.D., and Arrow Electronics for continued support of the DNALC's Biomedia addition. The Dolan DNA Learning Center was also able to contribute to the five-part television series on DNA and produce their *DNA Interactive* CD with a major grant from the Howard Hughes Medical Institute.

President's Council

Laboratory supporters who donated $25,000 or more to the research of Cold Spring Harbor Laboratory Fellows Drs. Lee Henry and Terrence Stick were invited to a weekend retreat May

President's Council birdwatching at Shelter Island.

17–18. The theme was "The Mind of the Bird," and Council members were treated to lectures on Saturday. This year, the topics were "How Birds Will Save the World" (John W. Fitzpatrick, Ph.D.); "The Wonders of Bird Migration" (Sidney Gauthreaux, Ph.D.); "Courtship and Mating in Birds" (Timothy Birkhead, Ph.D.); and "Developing Skills—Lessons from a Grey Parrot" (Irene Pepperberg, Ph.D.). On Sunday morning, participants went bird watching through the Mashomack Preserve on Shelter Island with our speakers, David Sibley (America's premier birder and best-known bird illustrator) and James Watson, who retains an interest in ornithology he developed early in life.

Planned Giving

Bill Miller, Chairman of the Board of Trustees, and his wife, Irene, generously committed a substantial charitable remainder trust which, upon maturity, will be used to fund Watson School fellowships. We also received a further payment from the Estate of Elisabeth S. Livingston. This gift has provided the means to build much-needed housing for postdoctoral researchers at Uplands Farm.

In the fall, the benefits of estate planning were the subject of two seminars for the general public, held at our Cancer Genome Research Center in Woodbury. The sessions were oversubscribed and stimulated additional interest in the Laboratory. In November, we were pleased to welcome Peter Stehli to spearhead the Laboratory's outreach for planned gifts, including bequests in wills, trusts, and other planned giving to benefit the education and research programs. A further meeting of the Cold Spring Harbor Laboratory Planned Giving Advisory Board was held in December. This board consists of accountants, tax advisors, trust officers, bank officers, investment advisors, investment bankers, and lawyers, representing firms located on Long Island, in New York City, and in Connecticut. The Board was briefed on the Laboratory's activities and accomplishments and provided much-appreciated advice in support of the Laboratory's goals.

Breast Cancer Groups

A crucial component to our breast cancer research program is the support we receive from hardworking local grassroots breast cancer groups such as Long Islanders Against Breast Cancer (L.I.A.B.C.); 1 in 9: The Long Island Breast Cancer Action Coalition; The Elisabeth McFarland Fund; and Long Beach Breast Cancer Coalition. This year, we were happy to become one of the beneficiaries of funds from F.A.C.T. (Find A Cure Today), a new fund-raising group based in Lloyd Harbor/Huntington. The Judi Shesh Memorial Foundation also made their first gift to us this year. We also gratefully acknowledge being chosen as one of the many recipients of funding from The Breast Cancer Research Foundation. The generous support we receive from these groups year after year is truly propelling our breast cancer research.

Alumni Association

This year, a Cold Spring Harbor Laboratory Alumni Association was formed, thanks to the efforts of Terri Grodzicker, Assistant Director for Academic Affairs, and the 18 alumni who agreed to serve on the board of the Alumni Association. The program aims to facilitate year-

round, ongoing relationships between Cold Spring Harbor Laboratory and the thousands of scientists who have worked or attended a meeting or course at the Laboratory. In this inaugural year, it is a particular pleasure to acknowledge the gifts of alumni committed to sustaining Cold Spring Harbor Laboratory as a special place for science.

Double Helix Gala and Exhibit

In honor of the 50th anniversary of the double helix, our generous supporters made additional contributions, which enabled us to present a celebratory gala at the Waldorf-Astoria on February 28. The Dana Foundation provided a lead gift early-on and major support followed from foundations and corporations, such as Forest Laboratories, Genzyme Corporation, Pall Corporation, Pfizer, The Albert and Mary Lasker Foundation, and The Alfred P. Sloan Foundation. An historical exhibit which highlighted the contributions of New York area research to the discovery of the DNA double-helix was held at the New York Public Library's Science, Industry, and Business Library from February until August. "Seeking the Secret of Life: The DNA Story in New York" was generously funded by The Camille and Henry Dreyfus Foundation, The Ellison Medical Foundation, Morgan and Finnegan LLP, and The Rockefeller Foundation.

Cultural Programs

A group of Laboratory benefactors generously supported our efforts during the past year to purchase a Steinway "B" piano for our concert series. Ownership of a piano will enable the Laboratory to defray the high costs of renting a piano for individual concerts and will allow for additional programming in the years to come. We thank Lucy and Mark Ptashne and the Jefferson Foundation; Roger Hugh Samet; Victoria and Anthony Sbarro; Douchet and Stephen Fischer for their gifts.

Concluding Remarks

The combined research and education programs at Cold Spring Harbor Laboratory are unique and have become a model for how research can drive innovation in science education. Close collaboration between our research and education programs allows the DNA Learning Center and our advanced science courses to benefit from the latest advances in research. The growth of these activities has been significant, but it is moderated by the desire to remain the best at what we do and provide the highest quality of education. To maintain our leadership position, we must increasingly rely on philanthropic support. For this reason, I am most grateful to those who have helped us this year.

Bruce Stillman
President and CEO

CHIEF OPERATING OFFICER'S REPORT

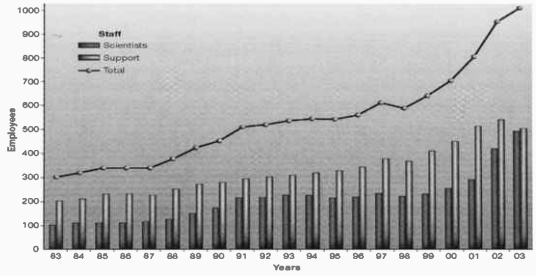
As with the prior year, the Laboratory experienced an unsettling blend of highs and lows in 2003. The academic and research programs continue to be unmatched in their excellence and in the promise they hold for solutions to major health problems. Endowment investment performance was outstanding after two dismal years in the equity markets. On the other hand, our operating results were disappointing.

It is easy to crow about the success of our various programs. The RNAi techniques developed by Greg Hannon and his colleagues are revolutionizing biology and are already providing pharmaceutical companies with RNAi libraries that target all human genes for therapeutic drug discovery in cancer and other areas. The research in neuroscience is equally as exciting as it branches into work on autism in addition to Alzheimer's disease and learning and memory. The success of the Watson School and its mission of providing a unique and accelerated Ph.D. experience has been validated in convincing fashion with the recent theses defenses of two outstanding graduate students, Amy Caudy and Ira Hall, both of whom completed the program in less than four years. The Dolan DNA Learning Center continues to expand the reach of its innovative laboratory program for secondary and high school students at the same time that its biomedia group churns out award-winning educational Web sites. The Cold Spring Harbor Laboratory Press, a distinguished publisher of scientific books, journals, and manuals, released its first undergraduate textbook as part of a new effort in this area of publishing. Our meetings and courses programs are thriving in a competitive environment, and Cold Spring Harbor remains the educational destination of choice for biologists from around the world. It is by no means a stretch to say that Cold Spring Harbor Laboratory is the intellectual capital of the world in the biological sciences.

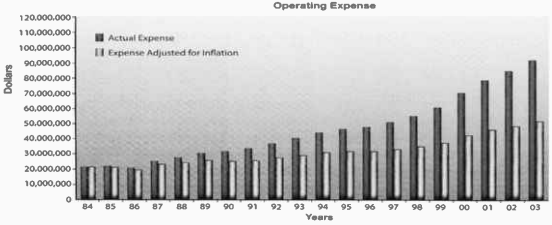
Forecasting the Laboratory's operating revenue is a challenging task due to the varied nature of the revenue streams and shifts in timing that are difficult to anticipate. The finance and grants departments take a meticulous approach to analyzing and forecasting all existing and pending grants from the public and private sectors. Hours are spent in this process with Dr. Stillman, who personally assesses the likelihood of a particular grant being funded and the timing of the research expenditures. Probabilities are assigned and the budget is built step-by-step. We analyze our technology transfer activities and assess the likelihood and timing of pending transactions. Judgments are made about new book sales and advertising revenue trends. Meetings attendance must be forecasted and is subject to the vagaries of variables such as SARS, terrorism, and inclement weather. Like all endowed institutions, we are also affected by the financial markets and interest rate fluctuations.

Coming on the heels of two years of rising expenses and shrinking endowment funds, 2003 was a difficult year for most educational institutions. Many high-quality universities reported budget deficits and layoffs. The goal at Cold Spring Harbor Laboratory has always been to balance our operating budget after full depreciation expense. We knew that this would be a difficult task in 2003, and, in fact, at the beginning of the year, we presented the Board of Trustees with a forecasted deficit of $970,000 after $5.4 million in depreciation expense. Expenses were closely managed over the course of the year. Administrative salary increases were capped at 2.5%, and capital spending from operations was kept well below deprecia-

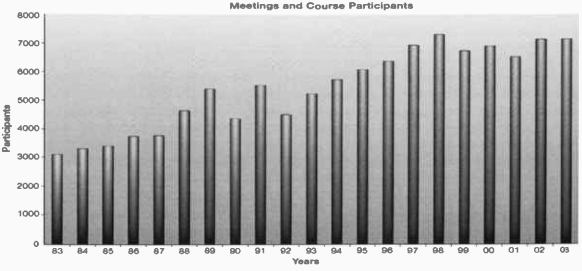
Full-time and Part-time Technical Support, Core Service, Publications, Meetings, Library, Public Affairs, Building and Grounds, Administrative, Personnel, Banbury Center, and Dolan DNA Learning Center



Operating Expense



Meetings and Course Participants



tion expense in order to preserve cash. Our hope at the outset was that we might exceed our grant revenue forecast and narrow the deficit by year-end. Unfortunately, things did not go our way. A major unanticipated blow struck when a large biotechnology company, which had been making substantial royalty payments on a Cold Spring Harbor patent for ten years, abruptly ceased payments claiming they no longer believed they were infringing the patent. With this, $1.2 million in revenue that would have dropped to the bottom line was gone, pending litigation, and $200,000 in unbudgeted legal expense was incurred. While we expect to prevail in the legal process and recover this income in 2004, it was a problem we did not need in 2003.

The other large variance from revenue affecting the year was only a matter of timing. The RNAi work referred to above has created substantial research support and licensing revenue opportunities that have been deftly negotiated by our technology transfer department. To date, agreements on this technology have been reached with OSI Pharmaceuticals and Merck. We had expected and budgeted to close a third agreement with Genentech in 2003. In fact, negotiations proved longer than expected and this revenue will not be realized until 2004.

The net effect is to report an operating deficit for the year of $2.0 million after depreciation despite the fact that total revenues were up 11% year over year to a high of $88 million. Although these results were largely event-driven, we are not satisfied and have taken action designed to avoid a deficit in 2004. At year-end 2003, a freeze on all administrative salaries and hiring was put into effect, and administrative department heads were asked to prepare to reduce budgets by 10% across the board. While it is painful not to award annual raises to such a dedicated workforce, we know that it is a necessary step for the time being. The Laboratory has a tradition of sound and conservative financial management that our Board of Trustees and our supporters have come to expect. Deficits of this magnitude must remain an exception to the rule.

The year was not without its bright spots. After two sub-par years, our endowment fund performed exceedingly well, outperforming the strong stock market by a wide margin. As the equity markets declined in the two prior years, the portfolio fell well below the equity/fixed income percentage guidelines established by our Investment Committee. Instead of the customary 60%/40% allocation, the portfolio shifted to 48%/52% early in 2003. Fortunately, the Committee, chaired by Lola Grace, had the courage to recommit to equities in March, and we reaped the benefits of a recovering stock market. Our three equity managers all outperformed the S&P allowing our equity portfolio to appreciate 39% for the calendar year as compared to 28% for the S&P. The fixed income investments also performed well, appreciating 5%—slightly ahead of the benchmarks. All in, our endowment funds grew nearly 26% for the year to a total of $224 million. Although pleased with these results, the Investment Committee has not lost sight of 2001–2002 and is not complacent. The Committee has recognized that our equity managers may be too highly correlated to one another, and an asset allocation and investment strategy review is in process.

The Laboratory continues to enjoy strong private support from friends in the local community. The Cold Spring Harbor Laboratory Association, led this year by Trudy Calabrese, does the all-important work of "friend-raising" and fund-raising for the Laboratory by organizing a myriad of events, lectures, concerts and dinners. The volunteer Directors of the Association, in concert with Trudy and CSHL Development Director Diane Fagiola, also manage the Annual Fund which provides the Laboratory with a critical source of unrestricted funds for research. We are pleased to report that this year, the fund exceeded its goal of $875,000 and crossed the $1 million milestone for the first time.

The Laboratory cannot realize its dreams and fulfill its mission without major private donations for capital projects. Last year, in describing plans for several new research buildings, I referred to an undisclosed gift toward "Phase I" of this project. We are deeply grateful to Mardi

Matheson and her late husband, William Matheson, for a substantial donation to advance the project, and we look forward to the day we dedicate the "William L. and Marjorie A. Matheson Cancer Building." The funding of this gift has allowed the Laboratory to go forward with the architectural and engineering plans for the project, work that is now very far along. Together with the $20 million pledge from Governor Pataki and New York State, more than 50% of the necessary funds have been raised. We are now in the process of applying to the Village of Laurel Hollow for final approval of these important plans.

Two of our valued trustees, Edward Harlow, Ph.D. and Lorne Mendell, Ph.D., had their terms expire at the end of the year. We are grateful for their years of service and will miss their participation and advice. We will also miss our dear friend and long time trustee Ed Marks who passed away in April (see Memorial on page viii). Fortunately, our Nominating Committee, chaired by Bob Lindsay, has done a terrific job in identifying and recruiting five outstanding new trustees to the board. We welcome David Botstein, Ph.D., James Stone, Alan Stephenson, Edward Travaglianti, and Roy Zuckerberg.

The Laboratory is blessed with an unbelievably talented and dedicated staff. They were given the opportunity to demonstrate their resourcefulness as well on August 14 when the widespread northeastern blackout caused us to lose power for 22 hours. This came in the midst of a meeting of 300 visiting cell biologists. With no stoves or elevators functioning in the kitchen and hundreds of mouths to feed, Jim Hope and the Food Service staff went to work by candlelight, retrieved the charcoal grills from the beach and prepared dinner as well as breakfast and lunch the next day on makeshift grills. In emergencies such as these, the freezers and refrigerators that preserve cell lines and other valuable research materials are powered by diesel-fired generators. However, there is storage for only an eight-hour supply of diesel fuel and beyond that, the situation can become dire especially when fuel vendors are also affected by the power outage. As the outage continued, Art Brings, Peter Stahl, and their colleagues in the Facilities Department convinced a local service station operator, whose pumps were down, to allow them to wire his pumps to a portable generator. Having accomplished this, our grounds crew shuttled back and forth to the Lab all night trucking tanks full of diesel fuel to the generators on the main campus. Over 2000 gallons of diesel was transported and burned before power was restored 22 hours later. We have become accustomed to seeing our staff rise to the occasion, under challenging circumstances, with an unparalleled degree of dedication and ingenuity.

No staff member has served the Laboratory with greater distinction than veteran leader Morgan Browne who announced his retirement after 19 years as Administrative Director and, most recently, Chief Financial Officer. During his tenure in these positions, Morgan guided the Laboratory through a period of rapid growth and expansion. Annual operating income over the period grew from $17 million to nearly $90 million. The endowment grew from $23 million to over $200 million, and the employee headcount increased from 320 to 900. Throughout his years here, Morgan consistently offered vision and sound judgment that served the Laboratory exceptionally well. While we will miss Morgan on a daily basis, we are pleased that we will continue to benefit from his expertise as he has agreed to stay involved through several committees related to the future expansion of Laboratory programs. Morgan's first "post-retirement" project is a historical documentation of the Laboratory's finances and endowment that appears on page 433 of this report. We are most grateful to Morgan for all he has contributed to Cold Spring Harbor Laboratory.

W. Dillaway Ayres, Jr.
Chief Operating Officer

50TH ANNIVERSARY OF THE DOUBLE HELIX

DNA 50 YEARS AGO AND TODAY

In Dublin, Ireland, just 60 years ago, the Austrian-born theoretical physicist, Erwin Schrödinger, gave a series of lectures under the title *What Is Life?* In them he made three important points. Firstly, why we grow up to be human as opposed to, say, a dog or tiger comes from information that is present in our chromosomes. Secondly, our genetic information is likely encoded in some digital way on stable molecules held together by covalent bonds. Thirdly, every time one chromosome becomes two chromosomes, their gene-carrying information molecules must be almost exactly copied.

Happily for me, Schrödinger transformed these lectures into a neat little book published by Cambridge University Press. I read it early in 1946 when I was 17 and a third-year student at the University of Chicago. It changed me from wanting to be a naturalist like Charles Darwin to becoming a geneticist searching for the chemical essence of the gene.

Just before I fell under *What is Life*'s spell, a chemical bombshell appeared for which Schrödinger had no forewarning—the molecule that carries genetic information is DNA (deoxyribonucleic acid). This major discovery was made in New York at The Rockefeller Institute by Oswald Avery, Maclyn McCarty, and Colin MacLeod. Their experiments, published in 1944, were almost foolproof. But most biologists took little notice of them. They remained wedded to the idea that proteins, being the most complex of biological molecules, should be the bearers of genetic information. In contrast, by the time I had my 1950 Ph.D. from Indiana University, I was obsessed with DNA, seeing the elucidation of its 3-D structure as the most important goal then before the biological world.


Oswald Avery


Maclyn McCarty

By then, the canny Scottish chemist, Alexander Todd, then working in Cambridge, England, had put the resources of his large laboratory toward establishing the covalent structure of DNA. Long before Avery's experiment, DNA was known to be a long polymeric molecule containing large numbers of four different building blocks—two purine nucleotides (A and G) and two pyrimidines (T and C). But how the nucleotide bases were linked together was not known until Todd and his co-worker Dan Brown established DNA's underlying 5′–3′ phosphodiester covalent bond. All DNA molecules possess the identical sugar phosphate backbones.


Colin MacLeod


Erwin Chargaff

Also seriously motivated by The Rockefeller Institute results was the Austrian-born biochemist Erwin Chargaff working at Columbia University. He reported in 1950 that the number of purine bases in DNA molecules was very similar to the number of pyrimidines. Even more important, the purine adenine was similar in amounts to pyrimidine thymine with the purine guanine similar in amount to pyrimidine cytosine. Equally crucial information came from

Photographs courtesy of the James D. Watson Collection, CSHL Archives and The Rockefeller University Archives

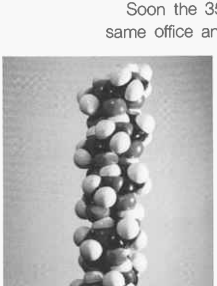
the Nottingham lab of the British physical chemist John Gulland. He found that purine and pyrimidine bases were located within DNA molecules in a way that prevented key hydrogen atoms from freely popping on or off. Not one of these three clever chemists, however, thought of themselves as going from their data to the 3-D arrangement of DNA atoms. It was beyond their field of expertise.

Sir Lawrence Bragg

The main experimental technique for working out the 3-D arrangement of atoms within molecules is X-ray crystallography, a field first populated by physicists. Originally, proceeding from X-ray diffraction patterns to a molecular structure was not straightforward. A year or two of sustained effort was usually required to see atomic locations of small molecules like amino acids and nucleotides. It then took much intellectual courage to pursue the structure of much, much bigger molecules like proteins and DNA. Leading the small postwar macromolecular crystallographic world was a tiny group of physicists and chemists at Cambridge University. Supported by the U.K. Medical Research Council, they worked at the Cavendish Laboratory under the patronage of its head, Sir Lawrence Bragg, the 1912 inventor of X-ray crystallography. Francis Crick had been on its staff for two years working on new methods to attack protein structure when I moved there in October 1951.

Linus Pauling

Soon the 35-year-old physicist Francis and I had desks in the same office and set ourselves the task of building 3-D molecular models of DNA. The great American chemist at Caltech, Linus Pauling, had six months before he used molecular model building to find the α-helical conformation of polypeptide chains without using detailed X-ray data. So we thought, why not extend this approach to DNA? Conceivably, sufficient experimental facts were known about DNA to let model building get to the answer without the need for further X-ray analysis. Already we knew from the King's College London lab that the DNA diameter was too big for a single-chain molecule. Francis' physicist friend Maurice Wilkins thought several chains were likely twisted around each other to form a multi-chained helix. Our first DNA model, conceived in late November, proved all too soon an embarrassment. It was incompatible with recent X-ray data obtained by Maurice's colleague, the Cambridge-trained physical chemist Rosalind Franklin. In our model, three sugar phosphate backbones formed a central core with the purine (A+G) and pyrimidine (T+C) bases facing outward. Rosalind told us that the backbone, not the bases, were on the outside.

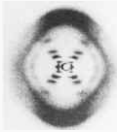


α-helical conformation of polypeptide chains

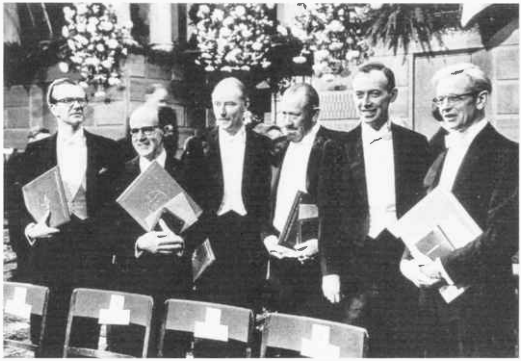
We only returned to model building after Linus Pauling made his own move on DNA. In December 1952, he devised a three-chain structure which incorrectly placed the purine and pyrimidine bases on the outside. When his manuscript arrived in Cambridge, Francis and I read it with great disbelief. Instead of scooping the Cavendish Lab, Pauling had fallen on his face by proposing that the chains were held together by hydrogen bonds between phosphate groups. In our minds, his structure defied the basic laws of chemistry. So Crick and I rushed over to Alex Todd's chemical laboratory to let him read Pauling's paper. He also decreed the Pauling model impossible.

Photographs courtesy of the James D. Watson Collection, CSHL Archives and Oregon State University Archives

Rosalind Franklin



Franklin's "B" form



Nobel Prize Ceremony, 1962

By this time, Crick and I were much better prepared chemically to move forward. The essence of DNA had to lie in how two, not three, intertwined chains were held together by hydrogen bonds between centrally located bases. Initially, we were held back by the wrong textbook positions of key hydrogen atoms on the bases guanine and thymine. Only a day passed between our knowing their correct locations and the finding of the Double Helix on February 28, 1953. Until that day we never thought we'd get the DNA structure by ourselves. We anticipated that help from the people at King's would be needed. To our relief, we soon learned that new experimental data fit our model perfectly.

The double helix instantly revealed the answer to the two key dilemmas posed by Schrödinger: How do you store and how do you copy genetic information? Instantly, we knew that DNA genetic information was digitally encoded through the order of the four bases (A, G, T and C) along DNA sugar phosphate backbones. In turn, DNA copying involves separating the double helix's two strands. The resulting single strands then serve as molds (templates) for the formation of second strands using the base pair rules—opposite an A you have to have a T and opposite a G you find a C.

Our resulting three (Watson and Crick, Wilkins and Wilson, and Franklin and Gosling) manuscripts went off to *Nature* on April 2, appearing in print only three weeks later on April 25. In our one page manuscript, we used a single sentence to say, "It has not escaped our notice that the spe-

RNA Tie Club

Photographs courtesy of Svenskt Pressfoto, Stockholm and the James D. Watson Collection, CSHL Archives

WATSON

(*Reprinted from Nature, Vol.* 171, *p.* 737, *April* 25, 1953)

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

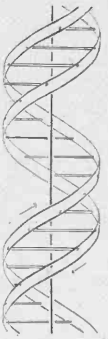
A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-ester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

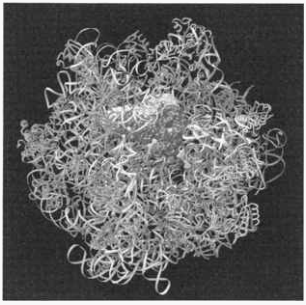
It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

Nature paper

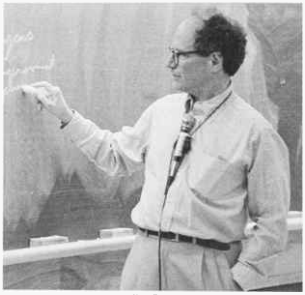
cific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." I then wanted to say nothing about how DNA is duplicated. It was so obvious we didn't have to say anything. But Crick said some people might think we were stupid and didn't see it. Soon after writing the first paper, we wrote a longer second paper, "The genetical implications of the structure of DNA." It came out in late May. Few reprints of it still exist, so it's more costly to buy than the first paper. I don't have any.

The 10 years following the finding of the double helix witnessed our learning how DNA sequence information is used by cells to make proteins the main molecular actors of cells. The two strands of the double helix temporarily separate to let single DNA chains be copied into RNA chains of complementary sequences using the same base pairing rules involved in copying DNA. The resulting single RNA chains provide the information for ordering amino acids with successive groups of three bases along RNA chains specifying successive amino acids along polypeptide chains. The specific groups of three bases which specify the 20 different amino acids comprise the Genetic Code. It is universally used by all forms of life from bacteria to higher plants and animals.



Harry Noller was the first to visualize the ribosome.

During protein synthesis, very large RNA containing particles known as ribosomes function as molecular factories which spin polypeptide chains in an assembly-line fashion. When we first worked on ribosomes, we thought their more than 100,000 atoms would preclude our ever knowing their precise structure at the 3-D level. Happily, a long succession of technical advances in X-ray crystallography recently let us see what they actually look like. The first scientist to visualize the ribosome in its entirety was Harry Noller at his lab in the University of California in Santa Cruz. There he observed the complete absence of protein molecules at the ribosomal site where peptide bonds are made. Atoms on RNA chains, not protein chains, enzymatically promote the linking together of the amino acids into polypeptide chains.



Wally Gilbert

Before our current DNA dominated world came into existence, there existed a simpler RNA world. How this RNA world arose we shall never know. No surveillance cameras witnessed how the first molecules of life arose. The earth's chemical components moreover have been constantly evolving and what carbon- and phosphorus-containing molecules existed when the RNA world started always will be a mystery. So there is no way to disprove the creationists' belief that life's origin was assisted by some God-like action. I don't believe this nor do almost all of my fellow biologists. As scientists, we only concern ourselves with ideas that can be experimentally tested. Truths only come from observations and experiments not from religious revelations.

Our principal concern now is to study life as it now exists after 2–3 billion years of Darwinian evolution. In doing so, we benefit from two major experimental advances of the 1970s. The first was the working out Recombinant DNA procedures which cut and paste DNA molecules. This powerful technology, devised just 30 years ago by Herb Boyer of UCSF and Stanley Cohen at Stanford let specific genes be isolated. Soon afterward, Wally Gilbert at Harvard and Fred Sanger at Cambridge devised rapid procedures

Gilbert photograph courtesy of the James D. Watson Collection, CSHL Archives

to read DNA messages. Until these DNA sequencing technologies became available in 1975 only cells, not humans, could read DNA messages of genes.

By 1988, improvements in DNA sequencing technology allowed the starting of the Human Genome Project. Its goal was to sequence the 24 very large DNA molecules present in each of the 24 different human chromosomes (a total of three billion base pairs). Before tackling the Human Genome itself, the genomes of a number of much smaller bacterial genomes were sequenced. Each such genome sequence reveals the precise number of genes involved in the growth and functioning of their respective bacteria. The smallest of these bacterial genomes code for only some 450 different proteins. With time, we will know the function of virtually every molecular actor in such simple bacterial cells. The life of a bacterium will then approach being as describable as a Swiss watch.

Obviously what we most want to understand is ourselves. Through the recent finishing of the Human Genome sequence, we now possess the location and exact structure of some 25,000 different genes. Using it, we have the potential to find out which combinations of the some 250 different human cancer genes are responsible for specific cases of human cancer. Equally explainable will be why and how some of us could come down with Alzheimer-like brain dementias. Over the next decade, most of the major genetic changes that lead to schizophrenia, bipolar diseases, unipolar depression, autism, and dyslexia will be found. Stopping many of these human tragedies is at last a realizable human goal for medical research. Though I'm already 75 years of age, I now have reason to hope that I may personally benefit from the Human Genome Project. When I helped start it 15 years ago, I thought that my children would be its first major beneficiaries.

Over a slightly longer time framework, improvements in DNA technologies will lead to the complete sequences for the biologically important portions of, say, 50,000 human genomes. This massive sequencing effort will let us find the specific DNA base sequences that give us much of our individual biological uniqueness. Why some of us in this room are left-handed or have premature white hair or have no wrinkles at 60 is due to our respective genes as is, say, much of our predisposition to being either thin or fat. Likewise many of our emotional differences, say, being predisposed to being happy as opposed to sad, in part will reflect differences in DNA sequences.

Increasingly, both what we as humans expect from ourselves and how we deal with other human beings will be affected by genetic knowledge. Assigning genetic causation to human capabilities and disabilities almost always has ethical consequences. Geneticists thus can no longer have a largely hands-off attitude to how their results bear on the functioning of human society. Likewise, moral philosophers, in their efforts to study the motivations behind human conduct, will best move forward by partnering with human geneticists searching for the genes that give to us our human nature. The Scotsman David Hume (1711–1770), in his profound "Treatise on Human Nature," said that humans are motivated by their passions (emotional feelings) as opposed to reason.

The main passion that led to the double helix was curiosity. All humans are curious to varying extents about something or other. I, Francis, Maurice, and Rosalind focused on DNA. There likely exist genetic reasons for why some of us seem to have so little curiosity and others so much. You naturally want your children to have lots of curiosity. With it they read books and learn about phenomena that need explanations. The passion for self-advancement also led to the Double Helix. Francis and I wanted to get there before Pauling. In his *Wealth of Nations*, the equally important Scots intellect Adam Smith argued that the freedom to work for your self-promotion promotes the well being of those about you.

Still highly argued today is the relative importance of nature and nurture in the expression of these human passions. Great differences in opinion exist, for example, as to whether

human concern for the well-being of other humans is an innate or a learned response acquired after birth. I come down on the side of nature through knowing that many acute observers of human behavior believe we help others largely because we anticipate that those helped in turn will help them. When I extend my hand to stop a nearby human losing his balance, I may be largely doing so because I was taught to so behave by my parents. Cultural instructions, however, cannot explain the feeding and caring of young birds by their parents. Such maternal (paternal) behavior ultimately must somehow go back to instructions encoded in their genes. The feeding and care by human mothers of their newborn children are likewise easiest understood as being gene-initiated.

Already back in the early 18th century, the enlightened Presbyterian minister Francis Hutchinson, whose career took him from Ulster to Dublin to Glasgow, argued that we are born with a good feeling toward others. In Aberdeen, the moral philosopher Thomas Read promoted a common sense approach to life basing it on his belief that humans are born with an innate sense of right or wrong. Soon these ideas of the 18th century Scotch Enlightenment flowed across the Atlantic to the British colonies giving to the American Revolution its intellectual underpinnings—human beings have innate needs for life, liberty, and the pursuit of happiness. To Tom Paine, common sense dictated that each human was entitled to make their own decisions and not receive them from kings or bishops whose authority did not come from those they ruled or presided over.

Common sense tells me that humans should proactively prevent as much genetic disease as science lets us. The rare mistakes in the copying of DNA information that make evolution possible of necessity leads to much individual genetic injustice. Random throws of the genetic dice during the formation of sperm and egg take away from too many infants the opportunity to participate in a meaningful life. Those unfortunate individuals genetically programmed, say, to have cystic fibrosis or muscular dystrophy represent unambiguous tragedies lacking in any compensatory side advantages. Common sense tells me that no one is seriously harmed by steps taken to prevent the birth of such children. Once born, their existences will of necessity generate many moments of anxiety, pain, and despair.

Common sense also dictates that prospective mothers should make the decisions as to what lives are worth their bringing into the world. Obviously, they should share their thoughts with the fathers, that is, if they are still about. Men, however, should have no veto over women's decisions. Few men will willingly share the burden of looking after tiny infants. Likely they are much less genetically programmed to give the necessary care. All such decisions like "Do I want to find out whether my child will be badly handicapped?" should be the choice of a woman. No one should force her either to be tested or not tested. But she should know what genetic tests exist and how respective genetic diseases, say, Down Syndrome or Fragile X disease will impact the future of those women that bear them.

How women will make these decisions will depend on their own sense of right and wrong and from what culture and religion they come out of. Great care must be taken to keep governmental and religious bodies from directly entering into genetics matters. We all know what happened in Nazi Germany when it decided what lives were not worth living. Of course, women occasionally will make decisions that they will later regret. Some of these may lead to governments, charities, and religious groups caring for children whose disabilities prove too great for their parents to handle. We should count on good common sense coming from most women, particularly those with prior exposure to genetic issues. The power to the people idea only really works when dealing with educated people. Unfortunately, current gene discovery now moves forward much faster than genetic education programs for girls and their mothers.

Common sense also dictates that no one should look at someone else's DNA without their explicit permission. I do not want others to know facts about my future that I myself do not

want to know. Until, say, we can stop the course of Alzheimer's disease, I do not want to find out whether I possess the Apolipoprotein E gene variant that increases your odds of being stricken with this dreadful dementia. Much harm to me might come from, say, my employer having DNA knowledge that I don't want to have.

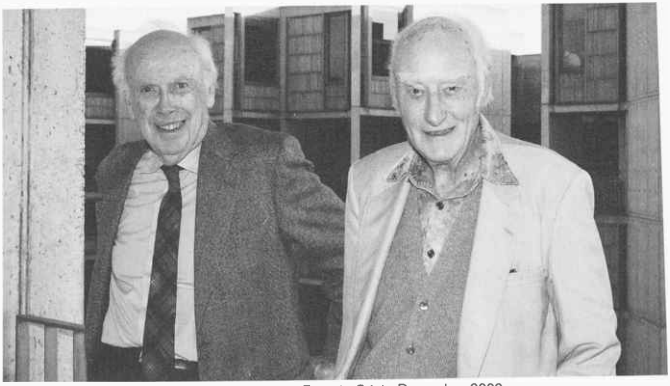
Over the next decade, there will come into existence more and more DNA tests which will help our personal physicians prescribe better medicines which will improve our lives. In giving them permission to so order such tests, it should be clear to all that no tests beyond those legally agreed to should be done without further permissions being given. There will of course be occasions of medical emergency when physicians should be allowed to go ahead on their own.

Common sense also suggests there will be increasing times when DNA knowledge about ourselves should be shared with others, say, our employers or insurance companies. There likely exist individuals who should not work with specific chemical agents that pose no great risk to most other human beings. Better to identify those at risk than to keep from human deployment agents that will advance our lives. When we seek large amounts of life insurance, we may find ourselves letting the respective companies look for DNA sequences that hint of how long we will live into the future. In the absence of such knowledge, they may have to assume that all of us are at high risk of early deaths. We must accept the fact that genetic privacy laws, by themselves, cannot negate much of the unfairness resulting from bad throws of the genetic dice.

We should also consider the still far off goal of improving human life by adding new genetic material to our germ plasma. I am in favor of so going forward though most of my fellow scientists, say, are afraid to do so. We don't yet know how to add genes safely, and why push before the public explosive issues that may never exist. But by adding an appropriate gene, we can improve the memory of mice. Why not the same with humans? To me it's common sense to so give us more effective brains. I don't see who we're offending by trying to so enhance ourselves. It goes against human nature to say that people should not try to improve the lives of their children and those that follow them.

James D. Watson

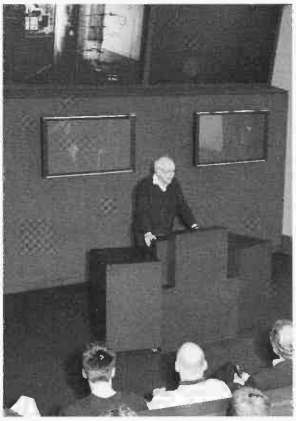
Delivered at the Commonwealth Club of San Francisco, October 9, 2003



James Watson and Francis Crick, December 2002

Photograph courtesy of Kent Schoeker, The Salk Institute

Cold Spring Harbor Laboratory's 50th Anniversary Celebrations



DNA at 50: Finding the Double Helix

DNA at 50: Finding the Double Helix

January 25 and February 8, 15, 2003
Dolan DNA Learning Center

Jim Watson gave three lectures on three separate days for a total of nine rare, public talks about his role in solving the structure of DNA, and then answered questions posed by the more than 900 people who attended these lectures. Visitors also had time to visit the Dolan DNA Learning Center's museum exhibit, "The Genes We Share," and purchase books authored by Dr. Watson.

Seeking the Secret of Life: The DNA Story in New York

February 24–August 29, 2003
New York Public Library–Science, Industry, and Business Library

The Library and Archives of Cold Spring Harbor Laboratory, The Rockefeller Archive Center of The Rockefeller University, and the New York Public Library's Science, Industry, and Business Library collaborated to present an exhibition which tells the story of the role played by individuals and organizations based in New York before, during, and after the 1953 discovery and presented an accompanying Web site, www.cshl.edu/CSHLlib/DNAinNY/index.htm. Among the items contributed by CSHL were a 1953 offprint of Watson and Crick's landmark *Nature* article, a photo of Watson and Crick with a large model they constructed of the DNA molecule, and a model of the Waring Blender Al Hershey used to separate phage from DNA. The project, initiated by Mila Pollock, Director, Library and Archives at Cold Spring Harbor Laboratory, was created by an Advisory Committee consisting of Pollock; Jan Witkowski, Director, Banbury Center at CSHL; Darwin Stapleton, Executive Director, Rockefeller Archive Center; and John V. Ganly, Assistant Director for Collections, The New York Public Library's Science, Industry, and Business Library, and their staffs.

Seeking the Secret of Life: The DNA Story in New York

Seeking the Secret of Life opening

Charlie Rose and Liz and Jim Watson at *DNA 50 Gala*

Stacy Blechinger, Barry Scheck, Marvin Lamont Anderson, Garnetta Bishop, Adele Bernhard, Peter Neufeld, and Joan Anderson at *DNA 50 Gala*.

Kitty Carlisle-Hart and Jim Watson at *DNA 50 Gala*

Nobel laureates at *DNA 50 Gala*

DNA 50 Gala

February 28, 2003
The Waldorf Astoria, New York City

An invitation-only, black-tie affair, scheduled 50 years to the day of the actual discovery by Watson and Crick, was co-organized by Charlie Prizzi, director of events and special projects, Cold Spring Harbor Laboratory; Columbia University; The Rockefeller University; and The Dana Alliance for Brain Initiatives to recognize not only the discovery by Watson and Crick, but also the pioneering research conducted in New York that led to the discovery. Charlie Rose hosted the evening, which included special appearances by Senator Charles Schumer, Deborah Norville (*Inside Edition*), and Tony Randall (*The Odd Couple*). Joel Grey (*Cabaret*) welcomed the more than 13 Nobel laureates and 700 guests who were in attendance with a rousing rendition of "Wilkommen" and the indomitable Kitty Carlisle Hart performed "Just One of Those Things" and "Here's to Life." Marvin Lamont Anderson, who with the help of Barry Scheck and Peter Neufeld, founders of "The Innocence Project" at Cardozo Law School, became the 99th person to be exonerated by DNA evidence, spoke of how his life was changed by genetics after he spent 15 years in prison wrongly accused of rape. The evening would not have been complete, however, without the special taped message from Francis Crick and Jim Watson's remarks on the discovery and its future impact.

The Biology of DNA

February 26–March 2, 2003
Grace Auditorium

(Right to left) P. Sharp, B. Stillman, J. Watson, and N. Zinder at *The Biology of DNA*

A four-day conference highlighting the past, present, and future of DNA science was organized by CSHL's David Stewart and Jan Witkowski. Entitled "The Biology of DNA," the meeting covered many topics relating to the biology of DNA, ranging from its replication, through the Genetic Code, to the human genome. Participants included a scientist from each of the 1953 DNA papers—James Watson, Herbert Wilson (who worked with Maurice Wilkins), and Ray Gosling (who worked with Rosalind Franklin), as well as Maclyn McCarty who, in 1945, with Avery and MacLeod, showed that DNA was the genetic material. Other participants included Nobel laureates Sydney Brenner, Thomas Cech, Walter Gilbert, Arthur Kornberg, Marshall Nirenberg, Phillip Sharp, and Hamilton Smith, and Francis Crick sent a special personal message via DVD. Other notable attendees were Sir Alec Jeffreys, inventor of DNA fingerprinting, and Matthew Meselson and Frank Stahl who, in what has been called the "most beautiful experiment in biology," showed how DNA is replicated.

The Double Helix 50th Anniversary Cultural Series

April–October, 2003
Grace Auditorium

The 2003 cultural series, organized by CSHL's Charlie Prizzi, honored Jim Watson and his creativity and cultural appreciation by bringing a wide variety of art, music, literature, and lectures to Cold Spring Harbor. Many of the lectures focused on the practical uses of the double helix and how it has benefited humanity for the past 50 years. Featured lecturers included Oliver Sacks, author of *Awakenings, The Man Who Mistook His Wife for a Hat,* and *Uncle Tungsten*; Barry Scheck, who formed "The Innocence Project" at Cardozo Law School and assisted in more than 100 cases where convictions were reversed or overturned based on DNA evidence; Eric Lander, one of the principal leaders of the Human Genome Project; and Robert Shaler, director of forensic biology for the New York City Medical Examiner's office, who has spent the last two years identifying more than 650 World Trade Center victims. The comprehensive line-up of concerts for the year also included an eclectic mix of musical performances; an art exhibit by Matthew Schreiber, Cold Spring Harbor Laboratory's 2002–2003 artist-in-residence; and a book signing by Jim Watson of his most recent book, *DNA: The Secret of Life*.

68th Cold Spring Harbor Symposium: The Genome of *Homo sapiens*

May 27–June 2, 2003
Grace Auditorium

Organized by Bruce Stillman and David Stewart, together with Jane Rogers and Eddy Rubin, the 68th Cold Spring Harbor Laboratory Symposium, *The Genome of Homo sapiens*, represented a remarkable book-

end to half a century of molecular biology. Fifty years prior, at a CSHL meeting in June 1953, Jim Watson made the first public presentation of the double helix model for the structure of DNA. In 1986, Jim organized a CSHL meeting on *The Molecular Biology of Homo Sapiens*, during which arguments for and against a proposal to sequence the entire 3-billion-letter human genome were presented. This year, the genome community—who have been meeting annually at Cold Spring Harbor every spring since 1988 to discuss ongoing progress and methods in genome science—were finally able to celebrate the completion of the finished sequence of the human genome (completed in Spring 2003) and to share in the discoveries arising out of this remarkable achievement, perhaps the first example of "big science" in biology. Dr. Francis Collins, who directed the United States' later efforts to sequence the human genome in collaboration with colleagues from around the world, gave the 2003 Dorcas Cummings Memorial Lecture about the human genome and the surprises scientists found as they sequenced it. This historic and notable Symposium attracted more than 530 participants from some 20 countries.

Spend a Day with DNA

July 12, 26 and August 9, 23, 2003
Cold Spring Harbor Laboratory, the Cinema Arts Centre (Huntington, New York), Heckscher Museum of Art (Huntington, New York)

The 50th Anniversary proved to be an excellent excuse to initiate a collaborative program with our neighbors: the Heckscher Museum of Art and the Cinema Arts Centre in Huntington. Although events commemorating the anniversary were occurring throughout New York and around the world all year, *Spend A Day With DNA* was truly an opportunity for Long Islanders to be a part of the festivities. More than 100 people from Cold Spring Harbor, Locust Valley, East Norwich, Glen Cove, Huntington, Northport, Roslyn, Port Washington, and Ontario, Canada attended. The program was the first-known attempt to relate how the discovery of DNA has served as an inspiration to artists and filmmakers throughout the world. Each of the four days included a tour of Cold Spring Harbor Laboratory; a visit to Cold Spring Harbor Laboratory's Dolan DNA Learning Center to explore its exhibition, *The Genes We Share;* a film at the Cinema Arts Centre; and a tour of *Genetic Expressions: Art After DNA* at the Heckscher Museum of Art. The program was sponsored by Vytra Health Plans.

Honest Jim: James D. Watson the Writer

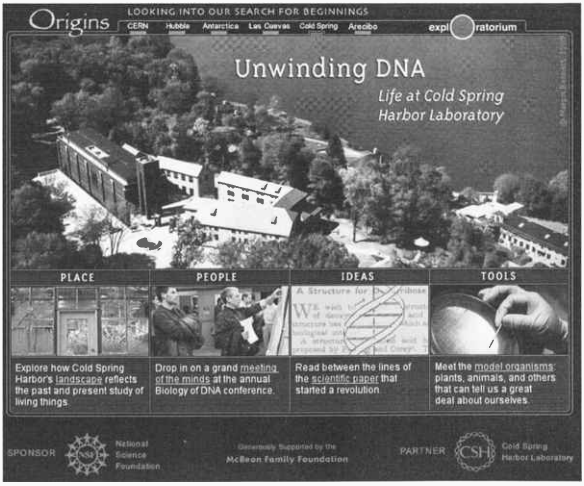
September 22–December 31, 2003
New York Public Library–Science, Industry, and Business Library

The second of two exhibits created by CSHL's Mila Pollock, Jan Witkowski, the Cold Spring Harbor library and archives staff, and a team from the New York Public Library, this exhibit focused on Dr. Watson as a writer, displaying his letters, theses, books, essays, lectures, photographs, and other memorabilia. A series of events accompanied "Honest Jim," including a lecture titled "My Life as a Writer," by Jim Watson and Andrew Berry, co-author of Jim's newest book *DNA: The Secret of Life*, and a very well-received panel discussion titled "Writing Science: Creating a Best-Seller." Panelists included Bruce Alberts, president of The National Academy of Science and author of *Molecular Biology of the Cell*; Robin Marantz Henig, author of *Monk in the Garden*; Sylvia Nasar, author of *A Beautiful Mind: The Life of Mathematical Genius and Nobel laureate John Nash*; Doron Weber, program director at the Sloan Foundation; and Jim Watson. The exhibit then moved to the University of Chicago.

Unwinding DNA: Life at Cold Spring Harbor Laboratory

Ongoing
www.exploratorium.edu/origins/coldspring/

The Exploratorium is a museum of science, art, and human perception housed within the walls of San Francisco's historic Palace of Fine Arts. Part of the Exploratorium's award-winning Web site, which hosts 12 million visitors per year, includes its *Origins* project. Through a collaboration with Peter Sherwood, Cold Spring Harbor Laboratory director of research communications, the Exploratorium launched a new *Origins* project called *Unwinding DNA: Life at Cold Spring Harbor Laboratory*. The project enabled the artists, writers, filmmakers, and producers of *Unwinding DNA* to explore many aspects of the history of Cold Spring Harbor Laboratory, the role of CSHL in the birth and growth of molecular biology, the 50th Anniversary of the double helix, and modern plant biology, genomics, and neuroscience research. In addition, through live and archived webcasts of one-on-one interviews and other dispatches issued during the "The Biology of DNA" meeting, the Exploratorium used *Unwinding DNA* to explain how scientific meetings fuel the process of discovery.



Unwinding DNA on Exploratorium Web site

Worldwide 50th Anniversary Celebrations

The year-long celebration of the discovery of the structure of DNA began with a private, celebratory dinner with Jim Watson and Francis Crick at the Salk Institute in January. Throughout the rest of 2003, a variety of symposia, lectures, and other special events were held, many of which Jim Watson attended. A sampling of these events is listed below.

EVENT	TITLE	LOCATION
SYMPOSIA		
Feb. 19–21	Time Inc: The Future of Life	Monterey, California
Feb. 26–Mar. 2	The Biology of DNA	Cold Spring Harbor Laboratory
April 7–11	World Life Sciences Forum	Lyon, France
April 8	Nobel's Conference: DNA's Day	Lyon, France
April 13	Linking the Double Helix with Health: Genetics in Nursing Research	Georgetown University, Washington, D.C.
April 14–15	From Double Helix to Human Sequence–and Beyond (hosted by NHGRI)	Natcher Conference Center, Bethesda, Maryland
April 15	Bringing the Genome to You	Smithsonian's National Museum of Natural History, Washington D.C.
April 16	Genetic Variation and Gene-Environment (G-E) Interaction in Human Health and Disease	National Institutes of Health, Washington, D.C.
April 16	Genes, Brain, Behavior: Before and Beyond Genomics	National Institutes of Health, Washington, D.C.
April 23–24	Royal Society Meeting: DNA Replication and Recombination	London, United Kingdom
April 25	DNA: 50 Years of the Double Helix	Cambridge, United Kingdom
April 22–25	A Celebration of the DNA Double Helix at 50	Trinity College Dublin
April 28–29	Royal Institution: Molecular Biology in the 20th Century	London, United Kingdom
April 28	DNA: 50 Years Ago and Today	Trinity College Dublin
May 27–June 2	The Genome of *Homo sapiens*	Cold Spring Harbor Laboratory
July 6–11	XIX International Congress of Genetics	Melbourne, Australia
Aug. 25–29	From the Lab to Everyday Life: Fifty Years of DNA	El Escorial (near Madrid, Spain)
Oct. 10–11	Genetech/Tularik Celebration: 50th Anniversary. Double Helix	University of California, Berkeley
Nov. 14–15	50th Anniversary Symposium	University of California, San Diego
EXHIBITS		
Feb. 6–April 11	From Code to Commodity: Genetics and Visual Art	New York Academy of Sciences, Gallery of Art and Science, New York
Feb. 22–Mar. 22	Women in Science (Genomically Yours)	Art Exhibit Universal Concepts Unlimited, New York
Feb. 25	PhotoGENEsis: Opus 2	The Museum at the Graduate Center, City University of New York

Feb. 28–May 25	*How Human: Life in the Post-Genome Era*	International Center for Photography, New York
March 1–31	*Brave New World*	Organization of Independent Artists, New York
April 25	*The Geeee! in Genome*	Ottawa, Canada

PUBLIC LECTURES

Feb. 2	Finding the Double Helix	Nature Biotechnology/ University of Miami Winter Biotechnology Symposium, Miami, Florida
Feb. 23	In the Round: A Conversation with Dr. James Watson	The Cooper Union for the Advancement of Science and Art, New York
March 3	Moving Forward with the Double Helix	New York Public Library, Science, Industry, and Business Library, New York
April 28	DNA: 50 Years Ago and Today	Trinity College Dublin
May 23	DNA at 50	Leipzig, Germany
June 4	DNA and Cancer	Express Scripts/Outcomes Conference, St. Louis
June 19	DNA and Our Futures	Pfizer, Groton, Connecticut
Aug. 5	From the Double Helix to the Human Genome Project	University of Michigan Biological Station
Sept. 23	From the Double Helix to the Human Genome Project	North Shore University Hospital, New York
Sept. 30	The Future of Medicine: From DNA to Angiogenesis	Angiogenesis Meeting, Cambridge, Massachusetts
Nov. 5	DNA: 50 Years Ago and Today	Youngstown State University
Nov. 11	The Discovery of DNA and Its Implications on Modern Medicine	AstraZeneca, Orlando, Florida

OTHER EVENTS

Ongoing	Origins—Unwinding DNA: Life at Cold Spring Harbor Laboratory (www.exploratorium.edu/origins/coldspring/)	Produced by The Exploratorium, San Francisco
January	An Uncirculated £2 Coin Commemorating the 50th Anniversary (designed by John Mills) Issued	British Royal Mint
Feb. 28	DNA 50 Gala	Waldorf-Astoria, New York
April	*DNA: The Secret of Life*, by James D. Watson and Andrew Berry Published	Book tour took Watson all over the United States and the world
April 25	National DNA Day	United States
May 14	*DNA: The Secret of Life* Film Premiere	Morehead Planetarium and Science Center, The University of North Carolina at Chapel Hill
August	*Inspiring Science: Jim Watson and the Age of DNA* Published	Cold Spring Harbor Laboratory Press
October 6	James D. Watson Institute of Genome Sciences Dedication	Zhejiang University, China


RESEARCH

See previous page for photos of the following scientific staff.

Row 1: Juana Arroyo; Nicholas Navin; Carine Becamel; Andreas Herbst; Rebecca Ewald; Michelle Cilia

Row 2: Christian Speck; Michael Deweese; Michelle Carmell; Francesco Roca; Jannic Boehm

Row 3: Farida Emran; Gordon Shepherd; Kendall Jensen; Gowan Tervo; Sunita Gupta; Sarah Newey

Row 4: Supriya Gangadharan; Michael Kwofie; Tsai-Ling Lee; Jonathan Sebat

Row 5: Stephanie Muller; Hong Jie Shen; Mary Byrne; Anindya Bagchi; Ping Hu; Inessa Hakker

Row 6: Ingrid Ehrlich; Jennifer Meth

Row 7: Takuya Takahashi; Jill Hemish; Khalid Siddiqui; Shanta Hinton

CANCER: GENE EXPRESSION

By identifying all the 40,000 or so proteins that govern human biology, the completion of the human genome sequence has ushered in a new age of biological discovery. Assigning functions to this myriad of proteins will involve several approaches. The lion's share of this functional characterization may well stem from "proteomics" or the study of how proteins are modified in the complex and dynamic environment of cells and tissues, and how they organize themselves into coherent, highly interactive networks that determine cell function. Michael Myers' lab is focused on identifying the global architecture of these protein networks. In particular, he is exploring the features of protein networks that make them robust (error-tolerant) as well as how such networks are altered in diseases such as cancer. These studies should provide key insights into which network components are promising targets for treating cancer.

Jacek Skowronski's lab focuses on a protein encoded by the genome of the virus that causes AIDS. The protein, called Nef, is an important determinant of HIV virulence and AIDS pathogenesis. By purifying human proteins that specifically bind to Nef, Skowronski's lab has identified several factors that are likely to control crucial events during AIDS pathogenesis. In so doing, they have uncovered potential new targets for therapies to combat this disease.

Leemor Joshua-Tor is collaborating with Greg Hannon and Rob Martienssen to determine the crystal structure and biochemistry of key protein complexes involved in RNA interference (RNAi), a promising tool for cancer gene discovery. In collaboration with Martienssen's group, Shiv Grewal's lab has shown how RNAi targets histone-modifying enzymes to specific regions of DNA, leading to the formation of an inactive heterochromatin structure. These studies have revealed an entirely unexpected connection between RNAi and DNA structure and have profound implications for understanding many aspects of gene regulation and the formation and stable inheritance of chromosome structure.

Like Joshua-Tor, Rui-Ming Xu focuses on determining the three-dimensional structure of proteins, at the atomic level, by using X-ray crystallography. Xu's lab focuses on proteins that carry out RNA splicing and on others that affect histone protein modification and thus the structure of DNA. By determining these protein structures, Xu's lab has uncovered several clues about how the proteins carry out RNA splicing and chromatin remodeling.

On the way from gene to protein, information passes through two RNA forms. The first is called a premessenger RNA transcript, which is a word-for-word translation of a gene's DNA. Premessenger RNA transcripts are then spliced to create mature messenger RNAs, the templates that cells use to make proteins. In many genetic diseases, gene mutations block RNA splicing. The resulting aberrant messenger RNAs lead to the production of abnormal proteins that cannot perform their functions properly. Adrian Krainer's lab has developed compounds that correct such RNA splicing defects. These compounds may ultimately be used as effective therapies for diseases as diverse as breast cancer, muscular dystrophy, and cystic fibrosis.

Nouria Hernandez and Winship Herr lead groups of researchers that explore the precise biochemical mechanisms by which DNA is transcribed into RNA. Both groups have looked at how specific protein-protein interactions control the action of enzymes called RNA polymerases, which produce RNA using the information coded in DNA. By examining how the herpes simplex virus prompts transcription and cell proliferation, Herr's group has found that a cellular transcription factor called HCF-1 acts at two key stages of the cell cycle and is now studying its role in processes that can lead to cancer.

William Tansey's lab studies the relationship between two processes that seem unrelated: transcription and protein destruction. They have found that factors from the protein destruction system are exploited in transcription, and hence actually help regulate genes. In particular, they are investigating how and why a potent transcription factor called Myc is destroyed, and how the loss of Myc protein destruction leads to cancer.

EPIGENETIC CONTROL OF GENE EXPRESSION

S. Grewal I. Hall K. Noma
S. Jia A. Malikzay
G. Xiao

After spending more than four years at Cold Spring Harbor Laboratory, I accepted a position at the National Cancer Institute (NCI), National Institutes of Health (NIH), in January this year and moved my laboratory to the Bethesda NIH campus. I enjoyed my stay here at CSHL and gained valuable experience thanks to many friends and colleagues and strong support provided by the Laboratory administration. An excellent and stimulating environment at CSHL greatly facilitated the rapid pace of our research. Our lab focused on the epigenetic control of gene expression and higher-order chromatin assembly. While at CSHL, we provided the first recorded evidence that Mendel's gene, in some instances, comprises DNA plus associated proteins. We also defined key molecular events critical for the assembly of higher-order chromatin structures. Studies from our lab and others demonstrated that specific posttranslational modifications of histone tails act in concert to establish a histone modification pattern, or "histone code," that signals for the establishment of heterochromatin, and dictate the specific organization of chromosomes into discrete structural and functional domains. More recently, our work provided an important link between RNA interference (RNAi) and heterochromatin assembly, a discovery that was selected "Breakthrough of the Year" by *Science* magazine. We continue to focus on the role of small RNAs generated by the RNAi pathway and histone-modifying activities in the dynamic regulation of higher-order chromosome organization that govern diverse cellular processes and have important implications for human diseases such as cancer.

RNAi AND HETEROCHROMATIN ASSEMBLY

The large genomes of higher eukaryotes suggest a need for stable packaging, particularly as most of the DNA does not code for proteins, and much consist of repetitious sequences, including remnants of invading transposons and other repeats. Studies from several organisms have shown that these repeat sequences are packaged preferentially into repressive heterochromatin complexes, which prohibit both transcription and recombination. It is largely believed that cellular defense mechanisms have evolved in higher eukaryotes to neutralize the invasion of these transposable elements by forming heterochromatin structures. However, the specific features of transposons that are recognized by the heterochromatin formation machinery are not fully understood. By using the fission yeast *Schizosaccharomyces pombe* as a model system, we recently showed that the RNAi pathway, in addition to its role in targeted destruction of mRNAs, is involved in initiating heterochromatin formation and silencing at repeated sequences. In this fundamentally novel process, RNA provides specificity to precisely target particular genomic loci for the recruitment of heterochromatin complexes. A key observation was that deletions of the factors involved in RNAi such as Argonaute (*ago1*), Dicer (*dcr1*), or RNA-dependent RNA polymerase (*rdp1*) in *S. pombe* cause defects in heterochromatin assembly. Specifically, the establishment of the heterochromatin-specific histone modifications such as methylation of histone H3 at Lys-9, and the targeting of HP1 homolog Swi6 to centromeres and the silent mating-type region of *S. pombe*, requires RNAi. Our analyses revealed that specialized repetitive DNA sequences and RNAi mechanisms cooperate to nucleate heterochromatin assembly. Furthermore, we discovered that, once nucleated, heterochromatin spreads to sequences surrounding the nucleation site in a Swi6/HP1-dependent manner, causing epigenetic silencing of nearby genes.

An important feature of the RNAi-mediated heterochromatin pathway is the generation of small interfering RNA (siRNAs) molecules of approximately 22 nucleotides from longer double-stranded RNAs by Dicer. Although siRNAs were known to be involved in this pathway, it was not clear exactly how these small RNAs promoted targeted assembly of heterochromatin. In our recent studies performed at NCI (in collaboration with the D. Moazed laboratory, Harvard Medical School), we have identified an RNAi effector complex called RITS (RNA-induced initiation of transcriptional gene silencing), which links siRNAs to heterochromatin assembly. RITS contains an Argonaute family protein, Ago1, a heterochromatin-associated chromodomain protein, Chp1, and a protein named Tas3 (Verdel et al., *Science 303:* 672 [2004]). In addi-

tion to the three protein subunits, RITS also contains siRNAs ranging from about 22 to 25 nucleotides. Collectively, our analyses suggest that double-stranded RNA generated from the repetitive sequences are processed into siRNAs. siRNAs join the RITS complex and guide the complex to homologous sequences, which involves pairing of siRNAs to nascent RNA transcripts or directly to DNA. The RITS-mediated recruitment of histone-modifying activities establishes a "histone code" for the nucleation of heterochromatin proteins such as Swi6/HP1. In the RNAi-independent spreading step, chromatin-bound Swi6/HP1 directly recruits histone-modifying enzymes such as a H3 Lys-9 histone methyltransferase to methylate adjacent nucleosomes, thus creating additional Swi6/HP1-binding sites. This process allows Swi6/HP1 and heterochromatin-specific histone modifications to spread in *cis* in a sequential manner, leading to the epigenetic silencing of genes surrounding the repeated sequences (see Fig. 1).

We also demonstrated that RNAi machinery is required for accurate segregation of chromosomes during mitosis and meiosis. Deletion of genes encoding Argonaute, Dicer, or RNA-dependent RNA polymerase causes a high incidence of chromosome missegregation. We found that chromosome segregation defects observed in RNAi mutant cells are due to loss cohesin at centromeres. Cohesins involved in sister-chromatid cohesion are preferentially enriched at *S. pombe* centromeres in a heterochromatin-dependent manner and have an essential role in the proper segregation of chromosomes during cell division. Our analyses also suggested the involvement of RNAi in proper regulation of chromosome architecture.

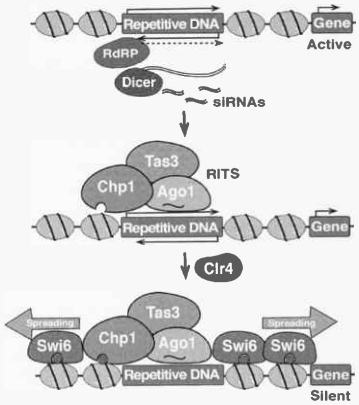


FIGURE 1 Model for RNAi-mediated targeting of heterochromatin. Double-stranded RNAs generated from repetitive sequences are processed by Dicer to generate siRNAs. siRNAs may serve as primers for RNA-dependent RNA polymerase (RdRp) to produce additional double-stranded RNAs from single-stranded transcripts. siRNAs join the RITS complex to provide specificity for localization of the complex to homologous sequences. The recruitment of histone-modifying activities, such as the Clr4 H3 Lys-9 histone methyltransferase, by the RITS complex creates a "histone code" for the binding of the chromodomain-containing proteins Chp1 and Swi6/HP1. The binding of Chp1 serves to stabilize RITS to the loci, whereas the binding of Swi6/HP1 results in heterochromatic spreading due to the combined activity of the H3 Lys-9 methylation and associated Swi6/HP1.

ROLE OF SIR2 HISTONE DEACETYLASE IN HETEROCHROMATIN ASSEMBLY

Hypoacetylated histones are a hallmark of heterochromatin in organisms ranging from yeast to humans. Histone deacetylation is carried out by both NAD^+-dependent and NAD^+-independent deacetylase enzymes. In the budding yeast *Saccharomyces cerevisiae*, deacetylation of histones in heterochromatic chromosomal domains requires Sir2, a phylogenetically conserved NAD^+-dependent deacetylase. In the fission yeast *S. pombe*, NAD^+-independent histone deacetylases are required for the formation of heterochromatin, but the role of Sir2-like deacetylases in this process had not been evaluated. In collaboration with the D. Moazed laboratory (Harvard Medical School), we found that spSir2, the *S. pombe* Sir2-like protein that is the most closely related to the *S. cerevisiae* Sir2, is an NAD^+-dependent deacetylase that efficiently deacetylates histone H3 Lys-9 and histone H4 Lys-16 in vitro. In cells carrying deletions of *sir2*, silencing at the mating-type region, telomeres, and the inner centromeric repeats (*imr*) is abolished, whereas silencing at the outer centromeric repeats (*otr*) and rDNA is weakly reduced. Furthermore, Sir2 is required for hypoacetylation and methylation of H3 Lys-9 and for the association of Swi6 with the above loci in vivo. In particular, we discovered that Sir2 appears to be dispensable for RNAi-mediated initial nucleation of heterochromatin at centromeric repeat sequences but has a crucial role in spreading of H3 Lys-9 methylation and Swi6 to regions surrounding the repeat sequences. Our findings suggest that the NAD^+-dependent deacetylase Sir2 has an important and conserved role in heterochromatin assembly in eukaryotes.

HISTONE ACETYLATION AND MAINTENANCE OF GENOMIC INTEGRITY

Distinct patterns of histone modifications established by histone-modifying enzymes control diverse chromosomal processes. Among these covalent modifications, the reversible acetyl modification at the lysine residues of histone tails is most well-studied. *S. pombe* contains three classical histone deacetylases, named Clr3, Clr6, and Hda1. Among these deacetylases, Clr6 is essential for viability. A conditional mutation in *clr6* also causes missegregation of chromosomes and ultraviolet light sensitivity. It has been hypothesized that Clr6 participates in global deacetylation of histones, affecting chromatin maturation throughout the genome. However, a detailed understanding of how Clr6 affects chromatin assembly and genome organization is lacking. To gain insight into the Clr6 function(s), we purified Clr6 and identified its associated proteins. We found that a chromodomain protein, Alp13, which belongs to the conserved MRG protein family linked to cellular senescence in humans, is associated with Clr6. In addition, Clr6 interacts with homologs of the mammalian transcriptional corepressor Sin3, Pst1, and Pst2, and a WD40 repeat-containing protein, Prw1. Alp13, Pst2, and Prw1 form a stable complex with Clr6 in the nucleus. Deletion of any of these factors causes progressive loss of viability, sensitivity to DNA-damaging agents, and impairs condensation/resolution of chromosomes during mitosis. This is accompanied by hyperacetylation of histones and a reduction in histone H3 Ser-10 phosphorylation that correlate with chromosome condensation during mitosis. Our analysis suggests that Clr6 and its interacting proteins are required for establishment of specific histone modification patterns, or "histone code," that are essential for fundamental chromosomal events, including gene regulation, recombination, chromosomal condensation, and segregation. Moreover, our results link the MRG protein family to histone deacetylation. Further analysis of the MRG protein family, as well as other HDAC components, may reveal fundamental mechanisms underlying genome integrity, cellular senescence, and aging.

HETEROCHROMATIN BARRIERS

We previously showed that heterochromatin complexes in *S. pombe* can spread along chromosomes and that specialized DNA elements called boundary elements protect the genes present in euchromatic regions from the repressive effects of nearby heterochromatin. At the mating-type region of *S. pombe*, two identical inverted repeats (IR-L and IR-R) surrounding a 20-kb heterochromatic domain prohibit heterochromatin spreading into surrounding regions containing genes. In collaboration with Dr. Amikam Cohen's laboratory (Hebrew University, Jerusalem), we characterized a novel gene named *epe1*, encoding a conserved nuclear protein with a jmjC domain, suppresses formation of heterochromatin in euchromatic regions. The jmjC domain of Epe1 is essential for its antisilencing acivity. Interestingly, Epe1 seems to disrupt heterochromatin assembly by modulating histone modification patterns.

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MECHANISMS OF TRANSCRIPTION

N. Hernandez F. Emran, P. Hu, B. Ma, S. Mayilvahanan, T. Oztaskin, K. Samudre, A. Saxena, Y. Sun, S. Wu, C.-C. Yuan

Gene expression is regulated to a large extent at the level of transcription. In eukaryotic cells, nuclear transcription is carried out by three types of RNA polymerases called RNA polymerases I, II, and III. Each type of RNA polymerase transcribes a specific set of genes. RNA polymerase II transcribes all the genes that encode proteins and is therefore the most extensively studied. Several years ago, RNA polymerase II transcription from a TATA-box-containing promoter was reconstituted in vitro with well-defined factors. Thus, at least in the case of a TATA-box-containing promoter, the factors required for RNA polymerase II basal transcription from a naked DNA template are known.

RNA polymerase III transcribes the ribosomal 5S RNA genes, transfer RNA genes, and other genes including the U6 small nuclear RNA (snRNA) gene. It recognizes three main classes of promoter structures. Classes I and II are present in the 5S RNA and tRNA genes, respectively, and are gene-internal. Class III is present in the U6 snRNA gene and other genes and is gene-external. The class III core promoters consist of two essential elements, a proximal sequence element (PSE) and a TATA box. Strikingly, in higher eukaryotes, the factors required for transcription from any of the classes of RNA polymerase III promoters have not been defined.

In previous work, we characterized two factors required for U6 transcription: the snRNA activating protein complex ($SNAP_c$), which is composed of five types of subunits and binds to the PSE, and Brf2-TFIIIB, which is composed of the TATA-box-binding protein (TBP), the TFIIB-related factor Brf2, and the SANT domain protein Bdp1, and binds to the TATA box. We also obtained a highly purified human RNA polymerase III fraction and characterized its composition. Besides RNA polymerase III subunits, the fraction contained spectrin, α-myosin, clathrin, α-actinin 4, HSC70, β-tubulin, β-actin, and calmodulin, as detected by mass spectrometry analysis, and perhaps traces of other factors not detectable by mass spectrometry.

In the past year, we combined recombinant $SNAP_c$, recombinant Brf2-TFIIIB, and the highly purified RNA polymerase III fraction and tested whether these factors were sufficient to direct transcription from the human U6 promoter. Strikingly, this combination of factors directed several rounds of accurately initiated and terminated transcription. Omission of any of the factors debilitated transcription, indicating that each factor was required, and transcription was as efficient as that directed by a crude extract from HeLa cells when normalized for RNA polymerase III amounts.

The result was surprising, because U6 transcription had been reported by other investigators to depend on an ill-defined factor called TFIIIC1. We therefore tested by immunoblot whether topoisomerase I, PC4, and NF-1, all of which had been reported to participate in RNA polymerase III transcription as part of the TFIIIC1 fraction, were present in our RNA polymerase III preparation, the only nonrecombinant factor in the transcription reaction. None of these factors was detectable, nor was the La antigen, which had been reported to be required for transcription termination and recycling of RNA polymerase III. This suggests that none of these proteins are required for U6 transcription in the reconstituted system.

To examine the role of the proteins other than polymerase III subunits detected by mass spectrometry in the highly purified RNA polymerase III fraction, we subjected it to one additional purification step over a "mini-Q" column. We found that of the factors other than RNA polymerase III subunits, β-actin, β-tubulin, and calmodulin were the only ones to peak with RNA polymerase III. Thus, these subunits appear to be strongly associated with RNA polymerase III and may have a role in transcription. On the other hand, spectrin, α-myosin, clathrin, α-actinin, and HSC70 were all separated from the peak of RNA polymerase III, indicating that none of these factors is strongly associated with the polymerase. Furthermore, we could not detect α-myosin, spectrin, HSC70, and clathrin in the active fractions, and it thus seems unlikely that any of these four proteins is required for transcription.

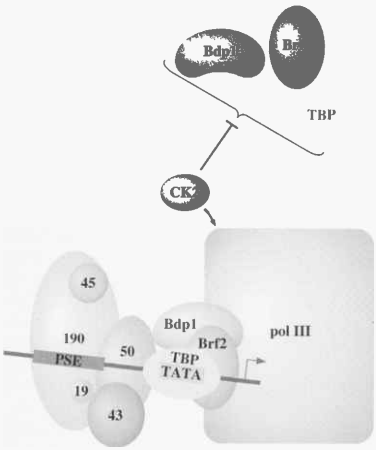

Figure 1 A minimal transcription system that directs several rounds of correctly initiated and terminated transcription from the human U6 promoter. $SNAP_c$ with its subunits SNAP190, SNAP50, SNAP45, SNAP43, and SNAP19 is shown bound to the proximal sequence element (PSE). Brf2-TFIIIB with its subunits TBP, Brf2, and Bdp1 is shown bound to the TATA box through the TBP subunit. $SNAP_c$ and TBP, and TBP and Brf2, are shown contacting each other because they bind cooperatively to DNA. CK2 phosphorylation of the polymerase III complex is required for transcription, whereas phosphorylation of the TBP-Brf2-Bdp1 factors is inhibitory.

We also tested the mini-Q fractions for the presence of CK2, which had been shown genetically to be required for polymerase III transcription in yeast. We found that traces of CK2 associate with RNA polymerase III. Furthermore, CK2 could be localized to U6 promoter sequences by chromatin immunoprecipitations. This suggested that CK2 may have a role in U6 transcription. Indeed, we found that CK2 phosphorylation of RNA polymerase III was required for transcription. On the other hand, phosphorylation of Brf2-TFIIIB was inhibitory. Together, these results show that, as summarized in Figure 1, transcription from the U6 promoter can be reconstituted in vitro with recombinant $SNAP_c$, recombinant Brf2-TFIIIB, and highly purified RNA polymerase III. Furthermore, they suggest that CK2 can regulate RNA polymerase III transcription both positively and negatively by phosphorylating different targets. The next challenges will be to identify the exact CK2 targets within the RNA polymerase III and the Brf2-TFIIIB complexes, to determine the role of these phosphorylation events in vivo, and to determine how CK2 is signaled to phosphorylate either RNA polymerase III and thus activate transcription or Brf2-TFIIIB and thus repress transcription.

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TRANSCRIPTIONAL REGULATION

W. Herr D. Aufiero, F. Bellouard, A. Bubulya, A.L. Chabes, H. Ding, E. Julien, Y. Liu, M. Mangone, C. Papazoglu, T. Tubon, C. Wu, J. Wysocka, X. Zhao

We all originate from a single cell that contains two complete sets of instructions—one from each parent—encoding how we should develop into a human being. Those instructions direct the cells to proliferate and at the appropriate time to differentiate into the approximately 250 different types of cells that make up an individual. Cells proliferate via repeated cycles of cell growth and division, and they differentiate by expressing specific sets of genes that permit them to perform specific functions. Both processes involve intricate patterns of regulation of gene expression, which often result from the regulation of gene transcription. In eukaryotes, the genes are packaged into chromatin, and the regulation of gene transcription involves the ability of transcriptional activators and repressors to direct changes in the structure of chromatin and to recruit one of three nuclear RNA polymerases—pol I, pol II, and pol III—to specific promoters.

Because viruses frequently target and reveal the roles of critical cellular regulators of host-cell processes, we use herpes simplex virus (HSV), a prevalent human pathogen, to probe transcriptional regulation in human cells. The infecting HSV virion contains a protein called VP16 that initiates the viral lytic program by activating viral gene transcription. Upon infection, VP16 associates with two cellular factors—a POU-homeodomain transcription factor called Oct-1 and the host-cell factor HCF-1—to form a transcriptional activating complex, called the VP16-induced complex. In recent years, our efforts have focused on (1) how the basal transcriptional machinery, including RNA polymerase, respond to transcriptional regulators like VP16; (2) the structure of the VP16-induced complex; and (3) the cellular functions of HCF-1. Below are described our studies reported this year.

ROLE OF THE INHIBITOR-OF-DNA-BINDING SURFACE OF HUMAN TBP IN RECRUITMENT OF TFIIB FAMILY MEMBERS

Historically, this project has made important contributions to our understanding of the mechanisms by which the basal transcriptional machinery responds to sequence-specific activators. Last year, we described the identification of an inhibitor-of-DNA-binding (IDB) surface on the human TATA-box-binding protein TBP. TATA-box recognition by TBP is a key step in transcriptional initiation complex assembly on TATA-box-containing pol II and III promoters. This process is inhibited by the IDB surface, which lies on the human TBP core domain (TBP_{CORE}), and is stimulated by promoter-specific basal transcription factors, such as two human TFIIB family members—the pol II factor TFIIB and the pol III factor Brf2, the latter being required for transcription from TATA-box-containing pol III promoters. In contrast, the third TFIIB family member Brf1, which is required for transcription from TATA-less pol III promoters, does not stimulate TBP binding to the TATA box.

Xuemei Zhao has now shown to our surprise that, in addition to its role in regulating TBP binding to a TATA box, the IDB surface is involved in TBP association with all three TFIIB family members. Interestingly, loss of IDB function has specific and diverse effects on each TFIIB family member. Indeed, the IDB and prototypical TFIIB-contact surfaces of TBP, which lie on opposite sides of the TBP_{CORE}, cooperate to form the wild-type TFIIB–TBP–TATA-box complex. These results reveal how, through differential usage of opposite surfaces of the TBP_{CORE}, TBP can achieve versatility in the assembly of pol II and pol III promoter complexes with the TFIIB family proteins.

PROTEOLYTIC PROCESSING IS NECESSARY TO SEPARATE AND ENSURE PROPER CELL GROWTH AND CYTOKINESIS FUNCTIONS OF HCF-1

Our studies of HCF-1 have been progressing rapidly and are leading to a realization that HCF-1 has independent roles in multiple aspects of cell proliferation. It turns out that HCF-1 is a highly conserved and abundant chromatin-associated protein. It exists as a heterodimeric complex of associated amino- ($HCF\text{-}1_N$) and carboxy- ($HCF\text{-}1_C$) terminal subunits that

result from proteolytic processing of a large precursor protein. Last year, we described studies using the temperature-sensitive tsBN67 hamster cell line showing that HCF-1 has a role in both G_1-phase progression and cytokinesis. In tsBN67 cells, the one X-linked copy of the *HCF-1* gene carries a proline-to-serine substitution at position 134 (P134S) in the HCF-1 Kelch domain, the region involved in VP16-induced complex formation and cellular chromatin association. This missense mutation does not grossly alter the stability or processing of HCF-1 at the nonpermissive temperature of 40°C, but it does induce HCF-1 dissociation from chromatin and a subsequent G_1/G_0-phase cell-proliferation arrest. The cell-proliferation arrest is accompanied by a conspicuous multinucleated (primarily binucleated) phenotype in about 15% of the arrested cells, indicating the associated cytokinesis defect. Both tsBN67 defects can be rescued by the HCF-1_N subunit and are suppressed by transformation with the DNA tumor virus SV40 early region, which has suggested that these two defects are connected.

This year, Eric Julien reported the use of small-interfering RNA (siRNA) to inactivate HCF-1 in an array of normal and transformed mammalian cells and thus to identify its cellular functions. Expanding upon a strategy pioneered by P. Lassus and Y. Lazebnik here at CSHL, Eric Julien designed recombinant siRNA-resistant forms of HCF-1 deletion mutants and prepared lines of HeLa cells stably expressing each deletion mutant. The results show that HCF-1 is a broadly acting regulator of passage through the G_1 phase and cytokinesis. Unexpectedly, given the close linkage found in tsBN67 cells, the G_1-phase and cytokinesis roles of HCF-1 are performed by separate HCF-1 subunits: the HCF-1_N subunit promotes passage through the G_1 phase, whereas the HCF-1_C subunit promotes proper cytokinesis. Furthermore, HCF-1 proteolytic processing is necessary for the ability of the HCF-1_C subunit to ensure proper cytokinesis. These results suggest that HCF-1 links the regulation of exit from mitosis as defined by proper cytokinesis and the G_1 phase of cell growth, possibly by coordinating reactivation of gene expression after mitosis.

HUMAN Sin3 DEACETYLASE AND TRITHORAX-RELATED Set1/Ash2 HISTONE H3 LYSINE 4 METHYLTRANSFERASE ARE TETHERED TOGETHER SELECTIVELY BY THE CELL-PROLIFERATION FACTOR HCF-1

This year, Joanna Wysocka, in collaboration with Michael P. Myers here at CSHL, reported more than 30 cellular proteins that associate with the HCF-1_N subunit in HeLa cells. The protein identities were revealed by mass spectrometry, and we reported an in-depth analysis of more than half of these proteins. These studies, in collaboration with Carol Laherty and Robert Eisenman (Fred Hutchison Cancer Research Center), showed how two separate regions of the HCF-1_N subunit that are both critical for its role in G_1-phase progression—the Kelch domain and a neighboring "Basic" region—associate with a previously uncharacterized human trithorax-related Set1/Ash2 histone methyltransferase (HMT) and the Sin3 histone deacetylase (HDAC), respectively. HCF-1 tethers the Set1/Ash2 and Sin3 transcriptional regulatory complexes together even though they are generally associated with opposite transcriptional outcomes: activation and repression of transcription, respectively.

The simultaneous association of HCF-1 with chromatin-modifying complexes apparently involved in both activation and repression of transcription was curious because, in the context of the VP16-induced complex, HCF-1 is only known to be involved in activation of transcription. We therefore asked which, if any, of these histone-modifying complexes is associated with HCF-1 when it is bound to VP16, as illustrated in Figure 1. We first prepared a HeLa cell line ectopically synthesizing epitope-tagged versions of VP16 (HA-VP16) and the HCF-1_N subunit (f-HCF-1_N) shown in Figure 1A. To identify VP16 association with the tagged HCF-1_N subunit and its associated factors, Joanna Wysocka first isolated tagged HCF-1_N-containing complexes by the two-step protocol that she had developed to isolate HCF-1_N complexes, performing a mock purification with VP16-only-containing HeLa cells in parallel. She then separated VP16-bound from VP16-free HCF-1_N complexes by VP16 immunoprecipitation as diagrammed in Figure 1B and analyzed corresponding amounts of the VP16-bound and VP16-free f-HCF-1_N fractions by immunoblot as shown in Figure 1C.

Consistent with VP16 association with the HCF-1_N subunit, the VP16 protein was present in the purified HCF-1_N subunit complexes. Furthermore, it was effectively recovered in the VP16 immunoprecipitation because all of the detectable VP16 was present in the VP16-bound and not in the VP16-free fraction (compare lanes 2 and 4, panel a). This VP16 immunoprecipitation step resulted in recovery of about 25% of the tagged HCF-1_N molecules (compare lanes 2 and 4, panel b), indicating that a significant portion of

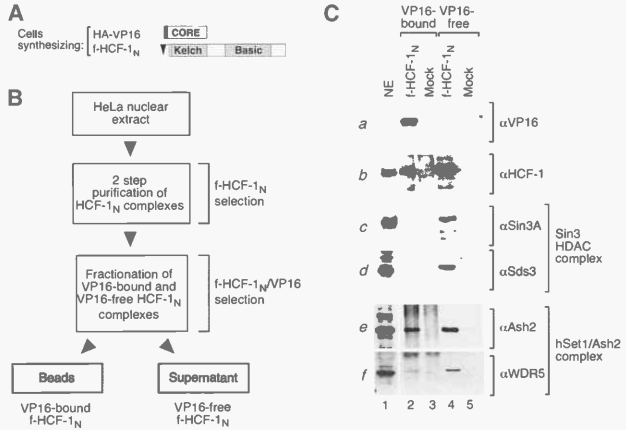

FIGURE 1 Preferential VP16 association with HCF-1_N-Set1/Ash2 HMT over HCF-1_N-Sin3 HDAC complexes. (*A*) Schematic of epitope-tagged VP16 and HCF-1_N molecules. (*B*) Schematic of the VP16-bound and VP16-free f-HCF-1_N complex fractionation. (C) Two-step HCF-1_N and mock-purified samples from HCF-1_N/VP16 and VP16 only cells were fractionated by anti-VP16 epitope tag immunoprecipitation into a VP16-bound HCF-1_N fraction (pellet) and VP16-free HCF-1_N fraction (supernatant) and analyzed by immunoblotting with VP16 (panel *a*), HCF-1 (panel *b*), Sin3A (panel *c*), Sds3 (panel *d*), Ash2 (panel *e*), and WDR5 (panel *f*) antibodies.

the HCF-1_N molecules in the cosynthesizing cells is associated with VP16. Of this portion, however, there is little, if any, coassociated Sin3A molecules or Sds3 molecules, both members of the repressive Sin3 HDAC complex (compare lanes 2 and 4, panels c and d). These results suggest that VP16 binds preferentially to HCF-1_N molecules that are not bound to the repressive Sin3 HDAC complex. In contrast, a significant portion of the activating Set1/Ash2 HMT complex members Ash2 and WDR5 cofractionate with the VP16-bound fraction (compare lanes 2 and 4, panels e and f). Thus, the prototypical transcriptional activator VP16 distinguishes between coregulatory complexes bound to HCF-1, favoring association with the histone-modifying complex implicated in activation of transcription.

In toto, these results have suggested that HCF-1 can broadly but selectively regulate transcription, both positively and negatively, through modulation of chromatin structure depending on which specific DNA-binding transcriptional regulator it is associated.

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STRUCTURAL BIOLOGY OF REGULATION OF NUCLEIC ACID REGULATORY MOLECULES AND PROTEOLYSIS

L. Joshua-Tor A. Auster, E. Enemark, C. Grunwald, H. He, T. Hu, P.R. Kumar, P. O'Farrell, S. Smith, J.-J. Song, N. Tolia, A. Tovkach, H. Zhou, R. Klajn (URP)

We study the molecular basis of cell regulatory processes combining the tools of structural biology and biochemistry to examine the molecular interactions associated with these processes. X-ray crystallography enables us to obtain the three-dimensional structures of individual proteins and their complexes with other molecules. We use biochemistry and molecular biology to characterize properties that can be correlated with protein structure, and we use information from molecular biology and genetics to study protein function.

Our current efforts center around two distinct themes. The first involves structural studies of protein complexes involved in nucleic acid regulatory processes. The second theme in the lab is the regulation of proteolysis. Here, we are studying caspase activation in apoptosis and a conserved family of oligomeric self-compartmentalizing intracellular proteases, the bleomycin hydrolases, which are involved in drug resistance.

DNA Binding and Assembly of the Papillomavirus Initiator Protein E1

E. Enemark, A. Auster [in collaboration with A. Stenlund, Cold Spring Harbor Laboratory]

Papillomaviruses are a large family of closely related viruses that give rise to warts in their hosts. Infection of the genital tract by the human papillomaviruses (HPVs) from this group represents one of the few firmly established links between viral infection and the development of cervical cancer, as HPV DNA is found in practically all cervical carcinomas. The E1 protein belongs to a family of multifunctional viral proteins whose main function is related to viral DNA replication. These proteins bind to the origin of DNA replication, melt the DNA duplex, possess DNA helicase activity, and recruit other cellular replication proteins such as DNA polymerase α and replication protein A (RPA). Most likely, different oligomeric forms of the two proteins are responsible for the different activities, and the sequential assembly of T antigen and E1 complexes ensures an ordered transition between these different activities. Ultimately, E1 forms a hexameric ring helicase on each strand that functions as the replicative DNA helicases that unwind the DNA in front of the replication fork.

In collaboration with Arne Stenlund's group here at CSHL, we embarked on structural studies to provide high-resolution structural information about E1, its DNA-binding activity and its assembly on DNA. These would provide general insight into the biochemical events that are involved in viral DNA replication, as well as a basis for the development of clinical intervention strategies. Second, the viral DNA replication machinery itself represents an obvious target for antiviral therapy, and detailed information such as high-resolution structures of viral proteins required for replication will greatly facilitate the development and testing of antiviral agents.

Previously, we solved the structures of the E1 DNA-binding domain from BPV both unbound and in two stages of assembly on the origin (dimer and tetramer). During the past year, we have extended our characterization of DNA binding by E1 to include human papillomavirus type 18 (HPV-18). HPV-18 is a high-risk strain of papillomavirus that causes cervical carcinoma, one of the most frequent causes of cancer death in women worldwide. In particular, HPV-18 causes adenocarcinoma, which is associated with poor prognosis. We determined the crystal structure of the monomeric HPV-18 E1 DNA-binding domain refined to 1.8-Å resolution. We demonstrated biochemically that the analogous residues required for E1 dimerization in BPV-1 and the low-risk HPV type 11 are also required for HPV-18 E1. We also found evidence that the HPV-18 E1 DNA-binding domain does not share

the same nucleotide and amino acid requirements for specific DNA recognition as BPV-1 and HPV-11 E1.

Mechanisms of RNAi

J.-J. Song, S. Smith [in collaboration with G.J. Hannon and R.A. Martienssen, Cold Spring Harbor Laboratory]

The introduction of double-stranded RNA (dsRNA) into a cell can trigger a gene silencing process called RNA interference (RNAi). This evolutionary conserved pathway, known also as post-transcriptional gene silencing (PTGS) in plants and quelling in *Neurospora*, is triggered either in response to dsRNA that is introduced exogenously, for example, by viral infection and targets endogenous RNAs for sequence-specific degradation, or by endogenous non-coding microRNAs (miRNA) which results in translational inhibition. The RNAi machinery has also been linked to the establishment of heterochromatin and proper centromere function in fission yeast by managing repetitive genomic elements, and it appears to have a role in development. RNAi has taken experimental biology by storm in recent years, since it has been greatly exploited as an ever-increasingly effective tool to study gene function by gene silencing through RNAi, not only in worms and plants where it was originally discovered, but now in many other systems including *Drosophila* and mammalian systems. Central to the RNAi machinery are two protein families, the Dicer and Argonaute proteins. Dicer, identified and characterized by Greg Hannon's laboratory here at CSHL as an RNase-III-type nuclease, cleaves dsRNA to generate short interfering RNAs (siRNAs) which are dsRNA of 19–24 nucleotides with two-nucleotide 3′ overhangs and a 5′ phosphate at each end. Dicer also cleaves short hairpin miRNA precursors which are in turn produced by a related nuclease called Drosha, to generate miRNAs. These structures, siRNAs or miRNAs, are incorporated into an effector complex called RISC, the RNA-induced silencing complex, to select its target. RISC contains an Argonaute protein as its signature component, which has two characteristic domains, PAZ, also found in Dicer family proteins, and PIWI.

Although there has been remarkable progress in unraveling the components of the RNAi machinery, we still do not understand how they work at the molecular level. Therefore, in collaboration with the groups of Greg Hannon and Rob Martienssen here at CSHL, we embarked on structural and biochemical studies of these proteins. We have determined the crystal structure of the PAZ domain of *Drosophila melanogaster* Argonaute 2 (Ago2-PAZ). The PAZ domain is composed of two subdomains that are oriented to form a cleft and resembles a baseball glove (see Fig. 1). One subdomain is a variant of the OB fold, a module that often binds single-stranded nucleic acids. Although PAZ shows only low-affinity nucleic acid binding, it appears to interact with the 3′ ends of single-stranded regions of RNA through the aromatic residues in the cleft. PAZ can bind to the characteristic two-nucleotide 3′ overhang of siRNAs, indicating that although it may not account for the binding affinity of Dicer or RISC, it may contribute to the specific and productive incorporation of siRNAs and miRNAs into the RNAi pathway.

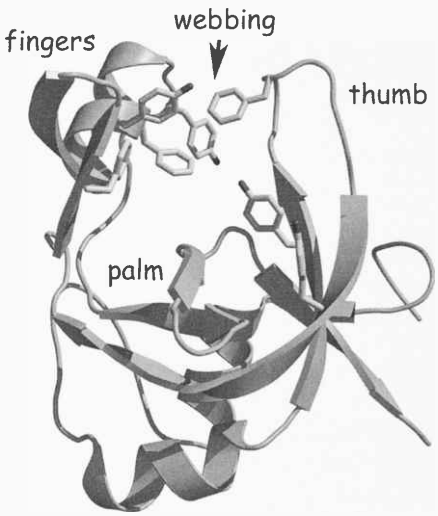

FIGURE 1 The PAZ domain resembles a right-handed baseball glove. Aromatic residues lining the intersubdomain cleft are involved in RNA binding.

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RNA SPLICING

A.R. Krainer E. Allemand, L. Cartegni, M. Hastings, R. Karni, L. Manche, H. Okunola, X. Roca, S. Shaw, P. Smith, Q.-S. Zhang, Z. Zhang

MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

RNA splicing is an essential step for the expression of most eukaryotic protein-coding genes. The selection and pairing of authentic splice sites within the spliceosome occur with extremely high fidelity, requiring precise interpretation of limited and dispersed sequence information present throughout introns and exons. In humans, approximately 75% of genes are expressed via alternative splicing, giving rise to multiple protein isoforms. The choice of alternative splice sites is commonly regulated to alter gene expression, either tissue-specifically or in response to a developmental program or to extracellular signals. The fact that multiple protein isoforms can be expressed from individual genes demonstrates that the classical "one gene–one enzyme" paradigm is no longer valid and provides an explanation for the unexpectedly small number of genes uncovered by genome-sequencing projects.

Both constitutive and alternative splicing mechanisms involve multiple protein components, as well as RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. The work in our lab focuses on the identification and molecular characterization of protein factors and sequence elements that are necessary for the catalysis and fidelity of splicing and/or for the regulation of alternative splice-site selection. We are interested in how the spliceosome correctly identifies the exons on pre-mRNA, and how certain point mutations in either exon or intron sequences cause aberrant splicing, leading to various genetic diseases. A summary of the studies that we have published in 2003 is given below.

SYNTHETIC MOLECULES THAT ACTIVATE EXON INCLUSION

On the basis of current knowledge about exonic splicing enhancer (ESE) elements and the SR proteins that recognize them to activate splicing, we have designed synthetic molecules that can be specifically targeted to weak exons to promote their inclusion (Fig. 1). This approach was dubbed ESSENCE (*E*xon-*S*pecific *S*plicing *EN*nhancement by small *C*himeric *E*ffectors). The synthetic molecules consist of an antisense moiety covalently attached to a peptide that acts as a splicing-activation domain. The antisense moiety binds to the target exon by Watson-Crick base pairing, so its sequence is chosen to be complementary to an exonic region of interest. The activation domain peptide is a simplified version of the natural RS domain of SR proteins, and we found that simple repeats of arginine-serine dipeptides are very effective. We have tested activator molecules targeted to exon 18 in *BRCA1* or to exon 7 of *SMN2*, genes that have a role in breast/ovarian cancer susceptibility and in spinal muscular atrophy, respectively. In both cases, the molecules restored correct splicing of transcripts from exon-skipping mutants in a cell-free system, and specificity was demonstrated by the fact that each molecule was only effective with the cognate target pre-mRNA. So far, we have used antisense moieties based on peptide–nucleic acid (PNA) chemistry, which offer the advantage of simple tandem synthesis with a peptide moiety, high stability, high target specificity and melting temperature, as well as resistance of the hybridized RNA target to RNase H. We found that longer RS peptides are more effective in promoting exon inclusion. The serines of the PNA-RS compounds undergo phosphorylation under splicing conditions, which together with the finding that the serines can be replaced by aspartic acid or glutamic acid, but not by alanine, indicates that an effective splicing-activation domain consists of alternating charges.

Three types of applications of these synthetic molecules can be envisaged. First, they can be used for therapeutic purposes, e.g., to correct exon-skipping defects due to mutations in a variety of genetic diseases. Second, they can be used to manipulate the patterns of alternative splicing of specific target pre-mRNAs, e.g., to study the functional consequences of switching from one isoform to another. Third, they can

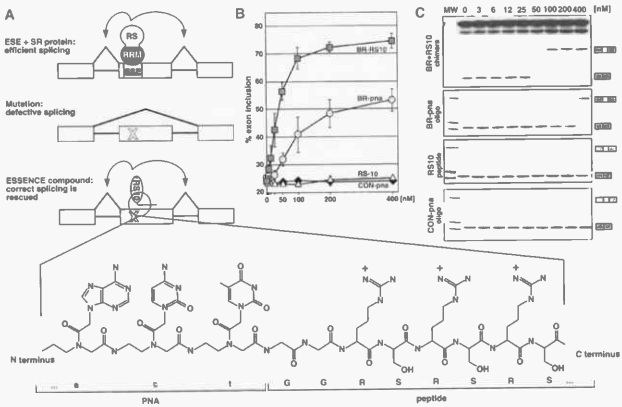

FIGURE 1 Synthetic compounds emulate the ESE-dependent function of SR proteins. (*A*) Diagram of compound design. An SR protein (*top*) binds to an ESE through its RNA-recognition motif (RRM) and promotes exon inclusion by recruiting the splicing machinery through its RS domain (RS). A point mutation within the ESE (*middle*) abrogates SR protein binding, and the exon is skipped. An ESSENCE compound (*bottom*) tethers a minimal RS domain to the exon by Watson-Crick base pairing and rescues splicing. Any sequence along the exon can be chosen as the target site. The chemical structure of a portion of the chimeric compound is shown at the bottom. It consists of a peptide with arginine-serine repeats linked to a targeting peptide–nucleic acid (PNA) oligonucleotide mimetic. The PNA moiety is at the amino terminus, and the recruiting peptide is at the carboxyl terminus to mimic the standard domain organization of SR proteins. A two-glycine linker separates the two minidomains to provide flexibility. Uppercase and lowercase letters indicate amino acids and nucleobases, respectively. (*B*) Dose-response curves showing effect of ESSENCE and control compounds on inclusion of exon 18 in transcripts of a mutant allele of *BRCA1*. (BR-RS10) A chimeric molecule consisting of a peptide with 10 RS repeats linked to a 12-nucleotide targeting PNA; (BR-pna) PNA with the same sequence but no peptide attached; (CON-pna) PNA of unrelated sequence; (RS10) peptide with 10 RS repeats. (C) In vitro splicing assays. Radiolabeled transcript with exons 16, 17, and 18, and introns 16 and 17, was spliced in HeLa cell nuclear extracts with the indicated concentrations of each compound. The alternatively spliced mRNAs resulting from inclusion or skipping of exon 18 were analyzed by denaturing polyacrylamide gel electrophoresis; quantitative data obtained by phosphorimage analysis were used in panel *B*. Efficient exon-18 inclusion requires both the peptide and exon-complementary PNA (BR-RS10); the PNA alone (BR-pna) gave a lower level of inclusion, comparable to what BR-RS10 gave at an eightfold lower concentration. A similar ESSENCE compound targeted to another gene (not shown), RS peptide alone (RS10), or PNA of unrelated sequence (CON-pna) had no effect on exon-18 inclusion.

be used to study the detailed mechanisms of splicing enhancement. Optimizing the delivery of these molecules into cells to test their effectiveness in vivo is now a major priority.

ANALYSIS OF EXONIC SPLICING ENHANCER MOTIFS

ESEs are *cis*-acting motifs that help guide spliceosome assembly to the splice sites flanking the exon in which they are present. As such, they contribute to exon definition, splicing fidelity, and alternative splicing regulation, and they are often the target of natural mutations that cause aberrant splicing, such as inappropriate exon skipping. Accurate identification of ESEs should facilitate the correct prediction of exons and introns in sequenced genomes, mechanistic studies of constitutive and alternative splicing, and interpretation of the effects of mutations in coding exons.

We are continuing to study ESE motifs recognized by particular SR proteins using a combination of experimental and bioinformatics methods (in collaboration with Jinhua Wang and Michael Zhang here at CSHL). ESEfinder (http://exon.cshl.edu/ESE/) is a Web-based tool that facilitates rapid analysis of exon sequences to identify putative ESEs responsive to the human SR proteins, SF2/ASF, SC35, SRp40, and SRp55, and to predict whether exonic mutations disrupt such elements. It provides quantitative measures and thresholds that reflect the ability of any sequence to enhance splicing in a defined exonic context, although not necessarily in its natural context. ESEfinder has proved to be especially useful for the analysis of coding-region mutations that cause exon skipping and to identify the splicing factors whose binding to the pre-mRNA is impaired by the mutations.

COMPARISON OF AUTHENTIC AND CRYPTIC SPLICE SITES

Cryptic splice sites are sequences that match the consensus 5′ splice-site or 3′ splice-site motifs, but which are used in splicing only when use of a natural splice site is disrupted by mutation. To determine the features that distinguish authentic from cryptic 5′ splice sites, we systematically analyzed a set of 76 cryptic 5′ splice sites activated by mutations in 46 human genes involved in various genetic diseases (in collaboration with Ravi Sachidanandam here at CSHL). These cryptic 5′ splice sites have a similar frequency distribution in exons and introns and tend to be located close to the authentic 5′ splice site. Statistical analysis of the strengths of the 5′ splice sites using the Shapiro and Senapathy matrix (a weight matrix based on nucleotide frequencies in natural 5′ splice sites) revealed that authentic 5′ splice sites have significantly higher score values than cryptic 5′ splice sites, which in turn have higher values than the mutant ones. β-globin provides an interesting exception to this rule, so we chose it for detailed experimental analysis. We found that the sequences of the β-globin authentic and cryptic 5′ splice sites, but not their surrounding context, determine the correct 5′ splice-site choice, although their respective scores do not reflect this functional difference. Our analysis provides a statistical basis to explain the competitive advantage of authentic over cryptic 5′ splice sites in most cases and should facilitate the development of tools to reliably predict the effect of disease-associated 5′ splice-site-disrupting mutations at the mRNA level.

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PROTEOMICS

M. Myers A. Kleiner L.L. Schmidt
G. Pegoraro C. van der Meijden
D. Perkowski

The completion of the human genome has ushered in a new age of biological discovery. This accomplishment has essentially identified all of the players governing human biology. The important work of assigning functions to this myriad of proteins has become one of the principal tasks of modern biology. Although many functions will be assigned using genetics or bioinformatics, the majority of this functional characterization will be performed by proteomics.

The goal of our laboratory is to understand how proteins and protein complexes regulate cellular behavior. In the environment of a cell, almost all of the proteins can be found in a highly interactive network. We focus on understanding the global architecture of this network and how this network generates robustness (error tolerance) and adaptability to the system and how this network is altered to produce and survive complex diseases, such as cancer.

Optimization of Protein Identification

D. Perkowski, L.L. Schmidt

Protein identification is the major tool of proteomics. We have been optimizing mass spectrometry (MS) for protein identification. MS has many advantages over other techniques for protein identification, especially its sensitivity. However, the improved sensitivity comes at a price, as the sample preparation becomes increasingly important to insure success. We have been optimizing a number of parameters to increase this success rate. Importantly, we have found that the method of staining the proteins prior to analysis can greatly affect the outcome of analysis, and we have begun to systematically explore different methods for high-sensitive protein visualization that do not adversely affect downstream MS analysis. We have found that fluorescent stains, such as SYPRO Ruby Red, give the greatest sensitivity without adversely affecting our analysis. However, we have found that negative zinc staining gives the best mix of protein visualization, ease of use, and MS sensitivity.

As part of the optimization, we have found that nanoscale chromatography gives the best sensitivity. Using convention chromatography media (packed beds of small, functionalized beads), we have been fabricating our own nanoscale (75-μm internal diameter) columns that operate at flow rates of 200–500 nl/min. The sensitivity and resolution of these nanoscale columns are related to their internal diameter. However, the internal pressures required for chromatography increase dramatically as diameter decreases, limiting the practical utility of miniaturizing conventional chromatography media. To overcome the pressure problem, we have developed a mesoporous, monolithic chromatography media that is well suited for miniaturization. The monolithic medium is composed of a single, highly cross-linked entity that is formed during the polymerization of silicas (sol-gels) or organic polymers (plastics). Initially, we incorporated octadecyl (C18) or octyl (C8) functional groups into our monoliths in order to create a reverse-phase resin. However, we discovered that monoliths composed of polymerized styrene and divinylbenzene created an exceptional reverse-phase medium that does not require further functionalization.

Monitoring Posttranslational Modifications

L.L. Schmidt, D. Perkowski

One of the challenges in the postgenomic era is to understand how proteins are regulated by posttranslational modifications. These modifications are responsible for controlling the activity of proteins and ultimately determining how a cell responds to its environment. Phosphorylation is considered to be the most common posttranslational modification, and its wide use as a regulatory mechanism has been attributed to its reversible nature. Alterations in protein phosphorylation have been shown to be the hallmark of many pathological conditions including cancer and diabetes. Therefore, efficient identification of phosphorylated proteins, as well as mapping the specific phosphoryla-

tion sites, has become one of the primary goals of proteomics. We have been attempting to develop sensitive and robust MS methods for characterizing this important posttranslational modification. Characterization of phosphorylated residues is complicated by three attributes of phosphorylation: (1) phosphorylation suppresses peptide detection by MS; (2) phosphorylation is generally substoichiometric; and (3) phosphorylated residues readily undergo neutral loss of the phosphoryl group. A number of groups, including our own, have found that the neutral loss can be used a diagnostic event to automatically trigger further analysis of a peptide. Our efforts have centered on developing reagents for the affinity purification of phosphorylated proteins and peptides. Initially, we tried immobilized metals as our affinity reagents, as this has been shown to interact strongly with phosphorylated residues. Using this technology, we estimate that 500 fmoles of phosphorylated peptide is required for characterization and that the major drawback of this technique is that the enrichment is highly contaminated by acidic peptides. To circumvent this, we will be adapting the styrene divinylbenzene polymers to create monoliths with high affinity toward phosphorylated peptides.

"Small World" Proteomics

C. van der Meijden, G. Pegoraro, A. Kleiner [in collaboration with R. Sachidanandam and J. Faith, Cold Spring Harbor Laboratory]

We have modeled the data from several high-throughput protein interaction screens as a network. We have chosen to model these data as a network because it is one of the only ways to make sense of these large and complicated data sets. In this model, each protein is treated as a node, and the interactions are treated as links between the nodes. In this way, the yeast protein network ends up looking very similar to the network of computers that make up the World Wide Web or the network of human social interactions that make this a "small world." On the basis of the network properties of the yeast protein network, we have been able to classify the yeast network as a scale-free network, in which only a fraction of the proteins are responsible for making the lion's share of the connections. One prediction from the network model is that these highly connected proteins are essential for yeast viability. In fact, we find that this is the case, as essential proteins are highly enriched in the pool of highly connected proteins. Importantly, not all highly connected genes are essential, and we are focusing on trying to understand the differences between these proteins and those that are essential. The overall goal of this study is to understand how these protein networks provide adaptability either to changes in environmental conditions or to genetic alterations, such as loss or duplication of a node (gene) that occurs during tumorigenesis.

We were hoping that our analysis of the yeast proteome network would reveal properties of mammalian networks and perhaps even how these networks get reprogrammed during tumorigenesis. One of the lessons from our work in yeast was that even this relatively small-scale network (6000 genes) was incredibly complex, suggesting that it would be difficult to make inroads into the tumor-specific network. We have chosen to take advantage of adeno-associated virus (AAV) to help us get a handle on the tumor-specific network. AAV has no known pathology in humans and has actually been described as having some tumor-suppressive capacity. We hope that an exploration of AAV-dependent tumor suppression would uncover important hubs in the tumor-specific network. Using MS, we have identified a number of host-cell proteins that associate with AAV-Rep68, an AAV protein required for viral replication. We have found that Rep68 associates with a number of proteins involved in DNA damage response (Ku70, Ku80, and DNA-dependent protein kinase). We have also found that AAV-Rep68 associates with ANP32B, a poorly characterized protein of uncertain function. Importantly, microarray data have demonstrated that ANP32B and AAV-Rep68 act synergistically to effect host-cell gene transcription.

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CELL SIGNALING IN HIV PATHOGENESIS

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Our interest lies in understanding the molecular mechanisms underlying the pathogenesis of AIDS and, in particular, understanding the functional consequences of interactions between viral proteins and the cellular regulatory machinery. The focus of our research is to understand the functions of Nef, a regulatory protein of human and simian immunodeficency viruses (HIV and SIV) that is an important determinant of virulence. We directed a major effort toward the identification of mechanisms and downstream effectors that mediate the effect of Nef on protein sorting and signal transduction machineries, and our experiments have been focused in two areas. First, we continued to study the mechanisms that mediate the selective down-regulation of cell surface receptors by Nef via the AP-2 clathrin adapter pathway. We found that this involves cooperative interactions between Nef, clathrin adapters, and cytoplasmic domains of target cell-surface receptors. This likely provides a general mechanism for selective cargo recruitment to the sites of endocytosis by this small viral protein. Second, we have focused heavily on the isolation and identification of cellular proteins that mediate the effects of Nef on signal transduction and endocytic machineries. This has led recently to the purification and microsequencing of several cellular factors that associate with Nef in T lymphocytes. Among them are factors that control critical events in the regulation of protein sorting, signal transduction, and gene expression. We have begun to verify selected novel interactions and to address their relevance to previously known and possibly novel functions of Nef, as well as to AIDS pathogenesis. Importantly, we found that Nef targets a critical molecular switch that regulates Rac GTPases downstream from chemokine- and antigen-initiated signaling pathways. This interaction enables Nef to influence multiple aspects of T-cell function, and thus provides an important mechanism by which Nef impacts pathogenesis by primate lentiviruses.

IDENTIFICATION OF PROTEINS ASSOCIATED WITH NEF IN T LYMPHOCYTES BY MASS SPECTROSCOPY

Nef is a small adapter protein that acts through associations with large protein complexes. One such example is that the complex(es) mediates the effects of Nef on the protein-sorting machinery at the plasma membrane and contains Nef and the AP-2 clathrin adapter, we described previously. Another example is the poorly characterized large complex(es) containing Nef and p21-activated kinase (PAK) kinase. We have been interested in idcntifying the targets of Nef in such complexes as well as additional downstream effectors of Nef that have escaped detection by previous studies. The unbiased approaches that have been used so far to identify immediate downstream effectors of Nef have relied heavily on the ability to reconstruct the relevant interactions in heterologous or in in vitro systems, including the yeast two-hybrid interaction screen (YTH) and chromatography of T-cell extracts on Nef-affinity columns. Thesc approaches have correctly identified a subset of Ncf ligands but have been used cxhaustively. Therefore, to bypass the limitations of the previously used methods, we purified to near homogeneity Nef and its associated protein complexes from T-cell lines stably expressing Nef, using an immunoaffinity purification protocol. With this approach, we detected a series of polypeptides with apparent molecular weights ranging from 20 kD to more than 250 kD that copurified with HIV-1 and SIV Nef proteins from a model human T-cell line. Next, we determined their sequences by mass spectroscopic analysis, in collaboration with Michael Myers at the CSHL Protein Chemistry Shared Resource. This led to the identification of sevcral common polypeptides associated with both 7.AH Nef and 239.AH Nef.

NEF BINDS DOCK2-ELMO1-RAC COMPLEX

Two abundant Nef-associated proteins were identified as DOCK2 and ELMO1. DOCK2 is a lymphocyte-specific CDM family (*C*ED5, *D*OCK180, *M*yoblast City) protein that regulates the activity of Rac1 and Rac2 GTPases downstream from chemokine receptors and the T-cell receptor (TCR) and is essential for lymphocyte migration and normal antigen-specific responses of T cells. Rac GTPases are members of the Rho subfamily of small GTP-binding proteins which control several processes including cytoskeletal

rearrangements during cell motility and T-cell activation. Recent studies showed that ELMO1 functionally cooperates with CDM family proteins to activate Rac. Significantly, our mass spectroscopic analyses of Nef-associated proteins also detected peptides shared by Rac1 and Rac2, raising the possibility that the ubiquitously expressed Rac1 and hematopoietic-cell-specific Rac2, which both regulate cytoskeletal dynamics and gene expression in T lymphocytes, also associate with HIV-1 Nef.

By analogy to previously described interactions between Rac, ELMO1 and CDM family proteins, our finding that DOCK2, ELMO1, and Rac2 copurified with HIV-1 Nef suggested that DOCK2 forms a ternary complex with ELMO1 and Rac2 and that Nef binds this complex. We investigated these possibilities by reconstituting these interactions in human embryonic kidney 293 (HEK 293) cells coexpressing ELMO1, DOCK2, Rac, and Nef each tagged with a different peptide epitope. We found that ELMO1 and Rac copurify with DOCK2, indicating that DOCK2 complexes with ELMO1 (DOCK2-ELMO1) and Rac. Furthermore, we found that ELMO1 and Rac also copurified with complexes containing both Nef and DOCK2. These data support the possibility that HIV-1 Nef binds DOCK2-ELMO1 complexes that contain Rac.

NEF ACTIVATES RAC IN RESTING HUMAN T LYMPHOCYTES

In nontransformed T lymphocytes, which are the major target of infection by HIV, Rac activation through DOCK2 is tied to chemotactic and antigenic stimuli. To assess whether Nef can uncouple these processes, we determined the effect of Nef on Rac activation in primary $CD4^+$ T lymphocytes in the absence of stimulation with antigen and chemokines. Resting T cells are normally refractory to productive infection by lentiviruses and lentivirus-derived vectors. However, a sizable fraction becomes permissive for infection when cultured in the presence of cytokines such as interleukin-7 (IL-7). We used this procedure to infect primary resting $CD4^+$ T lymphocytes with an HIV-1-derived vector expressing HIV-1 NA7 Nef (H-NA7) or a control *nef*-deleted vector (H-D). *env*-defective, VSV-G-pseudotyped viruses were used in these experiments because HIV-1 Env protein may activate DOCK2-controlled signaling pathways through binding to chemokine receptors such as CXCR4. As shown in Figure 1A, between 13% and 15% of $CD4^+$ T cells were productively infected as revealed by flow cytometry analysis of CD4 expression on the cell surface and intracellular p24 Gag expression. Cell extracts were prepared from the infected populations and PBD-GST pulldown assays were performed to determine the fraction of activated Rac. Strikingly, infection with H-NA7 resulted in a readily detectable increase in the steady-state level of activated Rac (Fig. 1B). The activation of Rac was specifically due to the expression of Nef and not other viral gene products, as infection with the otherwise isogenic H-D virus did not increase PBD-GST reactive Rac. The effect of Nef was specific toward Rac, because the activity of CDC42 GTPase, which also

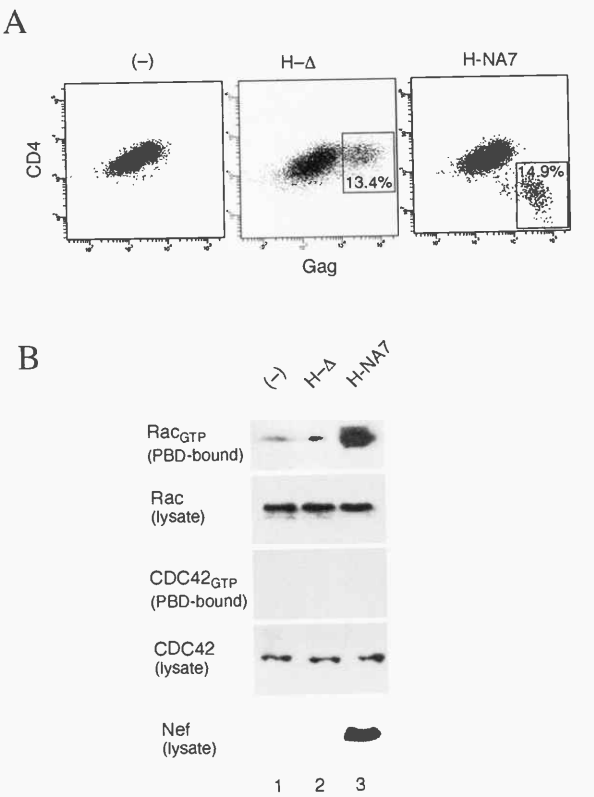

FIGURE 1 Nef activates Rac in resting $CD4^+$ T lymphocytes. (*A*) A flow cytometric analysis of Gag and CD4 expression in resting $CD4^+$ T lymphocytes transduced with HIV-1-derived vectors in the presence of IL-7. Percentages of cells productively infected with *nef*-deleted H-D vector (*inset in middle panel*), or with HIV-1 NA7 *nef* containing the H-NA7 vector (*right panel*) are shown. Results obtained with uninfected control $CD4^+$ T cells cultured in the presence of IL-7 are also shown (*left panel*). (*B*) HIV-1 Nef specifically activates Rac in resting primary $CD4^+$ T lymphocytes. Rac_{GTP} and $CDC42_{GTP}$ were precipitated with PAK1 PBD-GST from extracts prepared from $CD4^+$ T lymphocytes transduced with HIV-1-derived vectors, shown in *A*, and analyzed by immunobloting with α-CDC42- and a-Rac-specific antibodies.

uses PAK as a downstream effector, was not affected. Therefore, we concluded that Nef primarily activates Rac in $CD4^+$ T lymphocytes in the absence of antigenic stimuli.

Nef was reported to lower the threshold signal required for antigen-induced responses of T cells, and this effect was proposed to be an important component to stimulation of viral replication by Nef in vivo. Notably, Rac activation by DOCK2 facilitates T-cell responsiveness to antigen, as disrupted Rac activation in DOCK2(–/–) and Rac2(–/–) mice is associated with defective immunological synapse formation and depressed antigen-specific responses. Therefore, it is imperative to assess whether Nef promotes viral replication in T cells by activating Rac through association with DOCK2-ELMO1.

NEF INHIBITS T-CELL MIGRATION TO CHEMOKINES

DOCK2 regulates the activation of Rac proteins during lymphocyte migration in response to chemokine gradients. Therefore, we also asked whether Nef affects lymphocyte chemotaxis. Jurkat T cells, which constitutively express CXCR4, a major coreceptor for T-cell tropic HIV and a receptor for stromal-derived factor 1 (SDF-1), were transiently transfected with a control plasmid expressing enhanced green fluorescent protein (GFP) alone or with a plasmid that coexpresses Nef and a GFP marker protein from the same bicistronic transcription unit. We then measured the chemotaxis of transfected populations to SDF-1 using a trans-well migration assay.

Notable, we found that chemotaxis of cells coexpressing Nef and GFP, but not GFP alone, was inhibited in a dose-dependent manner. We also showed that (1) Nef causes a general defect in T-cell migration to chemokines and (2) the ability of Nef to inhibit T-cell migration is genetically linked to its ability to activate Rac via DOCK2-ELMO1.

One likely important consequence of inhibiting chemotaxis of the infected T cells is disruption of the immune response. The development and maturation of the immune response require the ordered migration of activated T cells to specific sites within lymphoid tissue, to drive B cells toward antibody production, isotype switching, and the affinity maturation of the antibody response. Since a large fraction of HIV-1-infected $CD4^+$ T cells are specific for HIV-1 antigens, this effect of Nef provides a new mechanism to suppress the antiviral immune response. Taken together, the functional interaction of Nef with DOCK2, ELMO1, and Rac enables Nef to modulate multiple aspects of T-cell function.

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TRANSCRIPTIONAL CONTROL AND THE UBIQUITIN-PROTEASOME SYSTEM

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The maintenance of normal cellular homeostasis requires that cells synthesize proteins when they are needed and destroy them when their function is no longer appropriate. These two "bookends" that define the life of any protein are, respectively, transcriptional regulation and ubiquitin (Ub)-mediated proteolysis. Despite the apparent dissimilarity of these processes, a growing body of evidence suggests that components of the Ub-proteasome system are intimately involved in regulating gene activity. Work in our laboratory takes advantage of the unexpected connection between the transcription and Ub-proteasome systems to achieve two objectives: To gain new insight into the actions of the oncoprotein transcription factor Myc, and to probe the depth with which the factors normally associated with protein destruction are involved in regulating gene activity.

INTIMATE CONNECTION OF THE TRANSCRIPTION AND UBIQUITIN-PROTEASOME SYSTEMS

Many transcription factors, particularly those involved in the control of cell growth, are unstable proteins that are destroyed by Ub-mediated proteolysis, a highly specific process in which the covalent attachment of Ub to target proteins signals their destruction by the 26S proteasome. Previous work in our laboratory has revealed that the destruction of transcription factors can be intimately connected to their ability to activate transcription. Specifically, we have found that the same domain in these proteins that allows them to activate transcription is responsible for their destruction. We have also found that, in some cases, transcription factors *need* to engage the Ub system and become ubiquitylated in order to function. This intimate connection between transcription factor activity and turnover has two important ramifications. First, it predicts that components of the Ub-proteasome system will be directly and intimately involved in the control of gene expression. Second, it reveals that if we study the destruction of transcription factors, we can learn not just about activator proteolysis, but also about activator function.

REGULATION OF Myc PROTEOLYSIS AND ACTIVITY

Myc is a basic helix-loop-helix leucine-zipper transcription factor that features prominently in the control of cell growth. Capable of acting as both a transcriptional activator and repressor, Myc controls the expression of genes required for cell growth and division. Consistent with the type of genes that it regulates, Myc is also is a major human oncoprotein that is related to approximately 70,000 cancer deaths in the United States each year. The expression and activity of Myc are tightly controlled at many levels, including via Ub-mediated proteolysis. We have previously shown that the destruction of Myc is connected to its activity as a transcription factor: Not only does the "transcriptional activation domain" (TAD) of Myc signal its ubiquitylation, but the ubiquitin-ligase Skp2—itself an oncoprotein—stimulates both Myc's transcriptional activity and its proteasomal destruction.

The interconnectivity of Myc function and destruction allows us to use the proteolysis of Myc as a tool toward understanding its activities. For example, we recently identified an element within Myc that is important for its rapid turnover. Unlike the amino-terminal TAD, this element does not appear to function by signaling Myc ubiquitylation, but rather by promoting the destruction of ubiquitylated Myc species. The involvement of this element—termed Myc box III (MbIII)—in Myc proteolysis prompted us to determine whether this element might also be involved in Myc activity. This analysis—performed in collaboration with Scott Lowe's laboratory here at CSHL—revealed that deletion of MbIII significantly reduced the ability of Myc to drive cellular transformation in vitro (Fig. 1). Because cellular transformation can be thought of as a balance between proliferation and apoptosis, we examined the ability of this mutant to promote cell division and to sensitize cells to apopto-

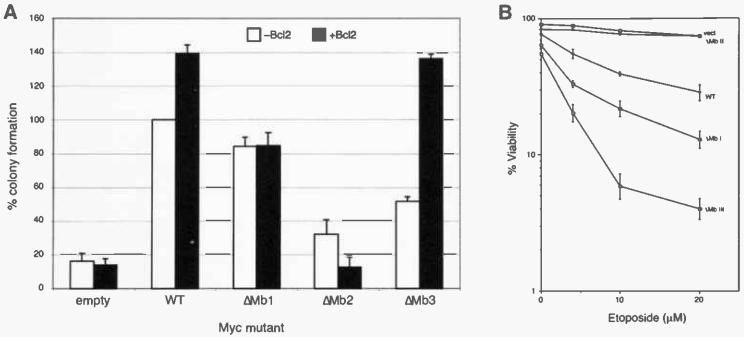

FIGURE 1 Loss of Myc box III decreases cellular transformation by enhancing the ability of Myc to induce apoptosis. (*A*) Analysis of Myc box deletion mutants in an in vitro Rat1A cell transformation assay. Rat1A cells were transduced with retroviruses expressing the indicated Myc proteins alone (*white bars*) or in conjunction with a virus expressing the anti-apoptotic protein Bcl2 (*black bars*). Cells were grown in soft agar, and colony formation was scored 21 days after plating. (*B*) Deletion of MbIII increases the ability of Myc to drive apoptosis in vitro. Rat1 cells expressing the indicated Myc proteins were treated with the apoptosis-stimulating drug etoposide. Cellular viability was scored by trypan blue exclusion. Cell death by apoptosis was confirmed by Annexin-V staining (not shown).

sis. Much to our surprise, we found that the ΔMbIII deletion mutant was as effective as wild-type Myc at driving cell division, but was substantially *more* active at stimulating apoptosis. To confirm that the decrease in cellular transformation by the ΔMbIII deletion mutant was indeed due to increased cell death, we inhibited apoptosis by expression of the anti-apoptotic factor Bcl2. As shown in Figure 1, inhibition of apoptosis rescued completely the transformation ability of the ΔMbIII protein, strongly suggesting that the only relevant difference between this mutant and wild-type Myc is the capacity to signal cell death.

Together, these data reveal that MbIII has an important role in regulating cellular transformation by Myc, and they support our notion that we can learn about Myc function from studying its destruction. Importantly, the data also reveal that Myc has an unexpected activity—the capacity to suppress its own ability to induce apoptosis. Thus, similar to strategies employed by a number of cancer-causing viruses (such as adenovirus), the Myc protein has evolved means to limit the cell death that results from the proliferative signal it provides. We speculate that this anti-apoptotic activity will be important for regulating tumor development in vivo, and may also represent a possible route for therapeutic intervention, by promoting apoptosis in cancer cells overexpressing the Myc protein.

UNDERSTANDING HOW THE UBIQUITIN-PROTEASOME SYSTEM CONTROLS GENE EXPRESSION

We are interested in understanding the fundamental mechanisms that connect the transcription and Ub-proteasome systems. In addition to studying the role of activator ubiquitylation and destruction in transcriptional control, we are also focusing on understanding how the proteasome itself can influence gene activity. In *Saccharomyces cerevisiae*, subunits of the 19S regulatory complex of the proteasome appear to have an important—and nonproteolytic—role in regulating gene expression. Indeed, it is curious to note that genes encoding two subunits of the 19S complex, *Sug1* (Rpt6) and *Sug2* (Rpt4), were first identified in a genetic screen for mutations that could suppress loss of the transcriptional activation domain of Gal4. Subsequent studies by Johnston and colleagues at UT Southwestern have shown that these effects are unrelated to Gal4 proteolysis, and that 19S proteins are recruited to a transcriptionally active *Gal1* gene in yeast. These findings suggest that the proteasome itself comes to chromatin and participates in some way in the transcription process.

To understand the role of proteasomal proteins in transcription, we first asked how the interaction of these proteins with chromatin is influenced by tran-

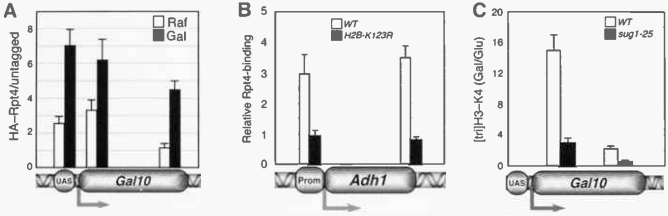

FIGURE 2 Recruitment of proteasomal ATPases to chromatin is linked to histone modifications. (*A*) Association of Rpt4 with the *Gal10* gene is modulated by gene activity. Yeast expressing HA-epitope-tagged Rpt4, or untagged control yeast, were grown in raffinose-containing media (30ºC), and either induced for 1 hr by growth in galactose-containing media (Gal) or uninduced by growth in fresh raffinose-containing media (Raf). ChIP was performed with an anti-HA antibody. Q-PCR was used to quantify association of Rpt4 with sequences corresponding to the *Gal10* promoter, as well as the promoter-proximal (–+100) and 3′ ends of the transcribed portion of the gene. (*B*) H2B ubiquitylation is required for recruitment of Rpt4 to the active *Adh1* gene. This experiment was performed as in *A*, except that mutant yeast carried a single point mutation in histone H2B (K123R) that blocks its ubiquitylation. Similar results were obtained when the gene encoding the Ub-conjugating enzyme for H2B was disrupted (not shown). (*C*) *The sug1-25* mutation disrupts inducible H3-K4 trimethylation at the *Gal10* gene. Wild-type (WT) or *sug1-25* yeast (containing a point mutation in the ATPase module of the proteasomal protein Rpt4) were grown in raffinose-containing media at 37ºC and induced for 1 hour by growth in galactose-containing media; ChIP was performed with antibodies against trimethylated H3-K4, or a no antibody control. Q-PCR was used to detect association of precipitated material with the 5′ (–+100) or 3′ ends of the *Gal10* coding sequence. *Gal10* gene induction was not disrupted by this mutation (*not shown*).

scription. We used chromatin immunoprecipitation (ChIP) to monitor the association of Rpt4 and Rpt6 with the *Gal10* gene under conditions in which it was either inactive or active. This analysis (Fig. 2) revealed that Rpt4 and Rpt6 associate with the inactive *Gal10* gene, but appear to be limited to a region immediately surrounding the promoter element. Upon *Gal10* gene activation, there is a pronounced redistribution of proteasome subunits throughout the transcribed portion of the gene. These data raise the possibility that proteasome subunits move either with or ahead of RNA polymerase II (pol II), and we are currently investigating this possibility.

To determine the significance of proteasome association with the *Gal10* gene, we next attempted to identify a signal that is required to recruit the proteasome to chromatin. Curiously, we found that proteasome binding to active genes is critically dependent on the ubiquitylation of histone H2B at lysine residue 123 (K123; Fig. 2). This finding was intriguing because ubiquitylation of H2B (Ub-H2B) is part of the "histone code" and is essential for signaling the methylation of histone H3 (Me-H3) at lysine residues K4 and K79. Ub-H2B/Me-H3 is an unusual example of the histone code because it involves modifications on two distinct histones (as opposed to modifications within a single histone "tail"). Nonetheless, like other modifications in the histone code, it is essential for establishing epigenetic patterns of gene expression. The methylation of histone H3 occurs at transcriptionally active regions of the genome, and likely establishes a barrier that repels the Sir proteins, restricting them to their sites on silenced genes. The observation that H2B ubiquitylation is also required to recruit the proteasome to chromatin led us to postulate that proteasomal proteins may function to stimulate H3 methylation. Indeed, we found that mutations in Rpt4 and Rpt6—but not other subunits of the proteasome—specifically disrupted H3 methylation at K4 and K79, but left H2B ubiquitylation intact (Fig. 2). Consistent with their effects on H3 methylation, mutations in these subunits also resulted in a widespread upregulation of silenced gene activity (data not shown). Together, these data suggest that proteasomal ATPases function to link the ubiquitylation of histone H2B to the methylation of histone H3. This link appears to be independent of the established role of the proteasome in protein destruction and is essential for the maintenance of normal patterns of silenced gene activity.

On the basis of our data, we speculate that ubiquitylation of histone H2B recruits the proteasome to the promoter of genes that have the capacity to be transcribed. When these genes are activated, we suggest that proteasomal proteins move with pol II and utilize

their protein chaperone functions (normally associated with the unfolding of proteasome substrates) to remodel the chromatin structure, permitting access of the Set1 and Dot1 methyltransferases to their target sites in histone H3. Regardless of the mechanism, however, we are excited to learn that subunits of the proteasome, which just a few years ago was thought of as an exclusively proteolytic machine, bind to chromatin and regulate the histone modifications that establish epigenetic gene control.

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William P. Tansey

STRUCTURAL BIOLOGY

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Y. Huang J. Min
Z. Li H. Shi

Our research focuses on understanding the mechanism and regulation of eukaryotic gene expression, at both the transcriptional and posttranscriptional levels. We use X-ray crystallography as our main tool to study the structure and function of key players in eukaryotic gene expression in atomic resolution detail. In particular, we continued to focus our attention on structural studies of proteins involved in mRNA processing and proteins that affect chromatin structure and dynamics. Our main accomplishments during 2003 include the following: (1) structure determination of human UAP56 (56-kD *U*2AF *a*ssociated *p*rotein) alone and in complex with ADP. UAP56, which contains a DECD RNA-dependent ATPase/helicase motif, is an essential splicing factor and a major nuclear export factor. (2) Solving the structure of the chromodomain of Polycomb in complex with a histone H3 peptide trimethylated at Lys-27. These two projects are described in detail below.

UAP56

The spliceosome is a complex molecular machine responsible for the removal of noncoding introns and the joining of exons. Although the two transesterification reactions of pre-mRNA splicing do not require ATP, the spliceosome is an energy-consuming machine. A number of ATP-utilizing enzymes are required for the assembly, remodeling, and disassembly of the spliceosome. More than a dozen spliceosomal proteins containing characteristic DExD/H ATPase/helicase motifs have been identified, yet no atomic resolution structure of any of the spliceosomal ATPase/helicases have been determined.

To discover the molecular mechanisms by which these spliceosomal "engines" work, we have determined the crystal structure of human UAP56 alone and in complex with ADP. Human UAP56 has an essential role in pre-mRNA splicing. The precise mechanism by which UAP56 functions in splicing is not known, although the characteristic DECD and other conserved sequence motifs are indicative of being an RNA-dependent ATPase/helicase. In addition to its function in pre-mRNA splicing, UAP56 also has important roles in the export of mRNA from the nucleus to the cytoplasm. Reduction of cellular UAP56 levels by RNAi (RNA interference) in *Drosophila* or *Caenorhabditis elegans* resulted in the retention of significant fractions of mRNAs in the nucleus. Similar results were observed in *sub2* (encodes a yeast UAP56 homolog) temperature-sensitive (ts) mutants in yeast.

UAP56 comprises two α/β fold domains (Fig. 1): an amino-terminal domain containing a seven-stranded parallel β-sheet with eight α-helices packed against the β-sheet on both sides, and a carboxy-terminal domain that also contains seven parallel β-strands but sandwiched between two α-helices on one side and

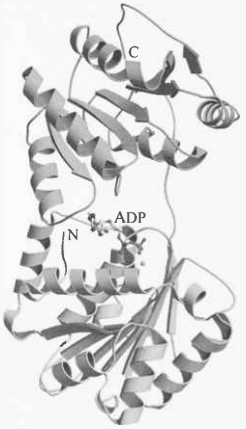

FIGURE 1 Crystal structure of UAP56 bound to Mg•ADP. The protein is shown as a ribbon diagram, and the magnesium ion and ADP are shown in a ball-and-stick model. The bottom lobe of the protein is the amino-terminal domain where ADP is bound, and the top lobe is the carboxy-terminal domain. The two domains are connected via a relatively flexible linker.

three helices on the other side. Both the amino- and carboxy-terminal domain structures resemble those of other RNA helicases exemplified by the structure of the yeast eukaryotic initiation factor 4A (eIF4A). Most importantly, the relative positioning of the two domains in UAP56 differs significantly from that observed in all other DExD/H-box ATPases/helicases. The binding of ADP does not introduce large domain movements, but the ADP-bound structure reveals that the P-loop in the Walker A motif undergoes transition from a "closed" to an "open" conformation to accommodate the binding of ADP. A comparison with the structure of single-stranded DNA-bound hepatitis C RNA helicase suggests that the ATPase activity of UAP56 may be stimulated by RNA bound to proteins that interact with UAP56, such as U2AF65, and UAP56 may function to displace proteins from the bound RNA during pre-mRNA splicing—a structure-based model that we are currently testing.

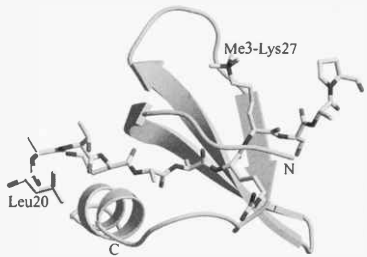

FIGURE 2 Structure of the Polycomb chromodomain in complex with a histone H3 peptide trimethylated at Lys-27. The chromodomain is shown in a ribbon representation and the histone peptide is shown as a ball-and-stick model.

POLYCOMB SPECIFICITY TOWARD HISTONE H3 METHYLATED AT LYS-27

Polycomb (PC) mediates the assembly of repressive higher-order chromatin structures in conjunction with the methylation of Lys-27 of histone H3 by a Polycomb group repressor complex. A similar mechanism in heterochromatin assembly is mediated by HP1, a chromodomain protein that binds to histone H3 methylated at Lys-9. The structure of the HP1 chromodomain in complex with a methyl-Lys-9 histone H3 peptide has been determined recently. The structure greatly advanced our understanding of methylated histone tail–chromodomain interactions. However, the HP1 structure offered no insights into the molecular mechanism of specific binding of the highly homologous PC chromodomain to histone H3 tails methylated at Lys-27.

To understand the molecular mechanism of the methyl-Lys-27 histone code recognition, we have determined a 1.4-Å resolution structure of the chromodomain of the Polycomb in complex with a histone H3 peptide trimethylated at Lys-27 (Fig. 2). The structure reveals a conserved mode of methyl-lysine binding and identifies Polycomb-specific interactions with histone H3. The structure also reveals a dPC dimer in the crystal lattice that is mediated by residues specifically conserved in the Polycomb family of chromodomains. The dimerization of dPC can effectively account for the histone binding specificity and provides new mechanistic insights into the function of Polycomb. We are currently pursuing further structural and biochemical characterization of Polycomb-histone H3 interactions aimed at pinpointing the precise determinants for Polycomb specificity.

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Cancer is caused by gene mutations. Mutations come in many forms: a change in the DNA sequence of a gene, the movement of a chromosome segment to an abnormal location, the complete or partial removal of a gene (deletion), or the addition of more than one copy of a gene (amplification). Michael Wigler and Rob Lucito have developed a method of unprecedented precision and power—called ROMA—for scanning the entire human genome to detect deletions and amplifications. By pinpointing regions where amplifications or deletions have occurred in DNA isolated from tumor biopsies, these researchers have identified dozens of candidate cancer genes from a variety of tumor types. Work by Masaaki Hamaguchi, Wigler, and Lucito has identified such genes in breast, ovarian, pancreatic, and other cancers. These genes are prime targets for the development of greatly improved diagnostic measures and more effective cancer therapies.

Scott Lowe continues to make important discoveries concerning the role of the *p53* tumor suppressor in programmed cell death, a natural process that rids the body of precancerous cells by triggering such cells to commit suicide. By developing powerful mouse models of cancer, and using RNA interference (RNAi) to explore *p53*'s functions, Scott and his colleagues have created a new paradigm for studying the heterogeneous responses of tumor cells to chemotherapy, with the hope of identifying improved cancer treatments. Working toward that goal, they have found a concrete example of a specific combination of drugs that can reverse resistance in tumor cells with a certain genetic makeup, thus highlighting a drug treatment strategy that might work for hard-to-treat cancers.

Alea Mills has developed a "chromosome engineering" system for creating mouse models of human disease. This approach allows her lab to generate mice with precise chromosome rearrangements that mimic alterations found in human diseases such as cancer. Mill's lab has also created a mouse model in which they control when and where a particular gene called *p63* is turned active, thus revealing the role of this important cancer-related gene in stem cell maintenance, cancer, and aging.

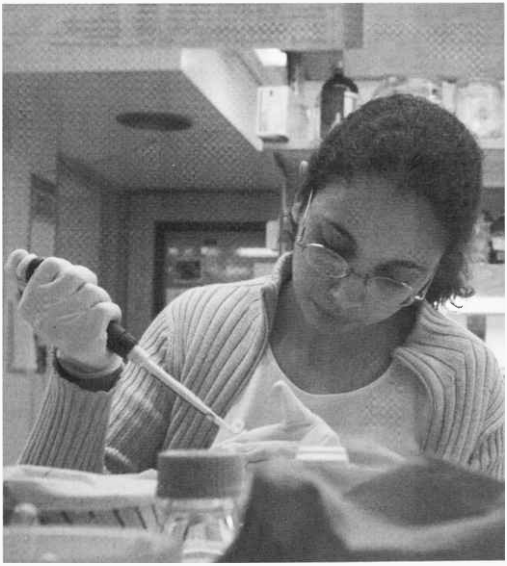
Greg Hannon and his colleagues have pioneered the study of RNAi as well as the application of RNAi to cancer research. RNAi leads to the silencing of gene expression, in part through the binding of small RNA molecules corresponding to particular messenger RNAs and the subsequent enzymatic destruction of the messenger RNAs (which blocks protein production). The researchers have discovered several of the molecules and mechanisms that carry out RNAi, including, recently, a protein that matches one that is silenced in a common form of X-linked mental retardation. They have shown that they can use RNAi to permanently silence the expression of virtually any gene in cultured cells, in living animals, or in organs reconstituted from stem cells (the latter in collaboration with Michael Hemann of Lowe's group). These findings have opened the door for them to use RNAi to discover new targets for cancer therapy, or to use RNAi itself as a therapy.

Through a process called angiogenesis, the *Id1* and *Id3* genes are involved in forming blood vessels that supply growing tumors with the nutrients they need. Therefore, *Id* genes and their targets are attractive candidates for antiangiogenic cancer therapies designed to kill tumors by cutting off their blood supply. Vivek Mittal's lab is using DNA microarray technology, RNAi, and mouse tumor models to identify antiangiogenesis targets. They recently identified two new pathways that, when obstructed, cut off tumor blood supply and have thus uncovered additional weak points that can be exploited for cancer therapy. Mittal's group has also created a genome-wide method for rapidly identifying the pieces of double-stranded RNA that are most effective at silencing any particular gene through RNAi.

Yuri Lazebnik is exploring ways to use the programmed cell death machinery to improve the efficiency and selectivity with which cancer treatments kill tumor cells. Paradoxically, although the hallmark of oncogenes is that they are associated with *causing* cancer, oncogenes also sensitize cancer cells to the lethal effects of chemotherapy drugs by inducing programmed cell death. This observation means that oncogenes might provide a way to induce the programmed cell death machinery specifically in cancer cells. Recently, Yuri's group has shown that chemotherapy activates an enzyme called caspase-

2, which in turn activates other caspases, leading to cell death. These findings reveal crucial information about how programmed cell death works, and how it might be used against cancer.

Approximately 1–2% of newborns have symptoms of sporadic genetic disease, or genetic disease that neither parent suffered from but rather arises de novo as a result of a genetic change in a parent's sperm or egg cell DNA. Eli Hatchwell's lab is developing methods for detecting subtle changes in DNA that cause such sporadic human genetic disease. In one study, the researchers have managed to detect a tiny deletion in human chromosome 2, which may be associated with mental retardation. The methods developed by Hatchwell's group should be useful for many applications, including cancer research.



Sihem Cheloufi, graduate student in the Mills lab.

TUMOR SUPPRESSOR GENES INVOLVED IN BREAST CANCER DEVELOPMENT

M. Hamaguchi N. Kobayashi R. Shudo
J. Meth V. Siripurapu

Our lab is interested in the discovery and characterization of cancer-related genes, especially tumor suppressor genes that are ablated in cancer cells. The identification of these genes and subsequent studies into their biological functions have provided vast new insights into the development of cancer. A great deal is now known about the relationship between cancer development and cellular functions. In the case of breast cancer, the isolation of tumor suppressor genes *BRCA1* and *BRCA2* has had an impact on several aspects of cancer treatments as well as cancer research. *BRCA1* and *BRCA2* genes can be utilized for identification of individuals at high risk of developing breast cancers. This has been crucial in the development of risk assessment. However, tumor suppressor genes responsible for sporadic breast cancer remain to be discovered. We have isolated a previously uncharacterized gene, *DBC2* (*d*eleted in *b*reast *c*ancer 2), that is likely to be a tumor suppressor gene for sporadic breast cancer. Activation of *DBC2* in a breast cancer cell line resulted in growth arrest of the cells, whereas naturally occurring *DBC2* mutants do not suppress tumor growth. The ultimate goal of our research is the elucidation of the tumor suppression mechanisms of *DBC2* and development of new cancer therapy based on the findings.

DBC2 and Gene Expression

M. Hamaguchi, V. Siripurapu [in collaboration with S. Powers, Tularik Inc.]

Clarification of the physiological functions of *DBC2* will provide clues for understanding the mechanisms of breast cancer development, since more than half of breast cancers were found to exploit inactivation of *DBC2*. To identify biological pathways that involve *DBC2,* the functions of cellular genes are monitored while *DBC2* activity is artificially altered. We apply two molecular biological technologies to control *DBC2* activity, an inducible gene expression system and RNA interference (RNAi). In the inducible gene expression system, *DBC2* is placed under a promoter that can be activated by an insect hormone, ecdysone or its homolog, Muristerone A. When the inducer is administered, *DBC2* will be expressed. The gene expression level can be controlled by altering the quantity of the inducer. Since these chemicals do not normally exist in mammalian cells, they will not affect expression of the other mammalian genes. We are studying the effects of *DBC2* expression in cancer cells by using this system. Reversely, *DBC2* expression can be knocked down by using RNAi. We have demonstrated that carefully designed short RNA fragments can inhibit *DBC2* translation effectively in various types of cells, resulting in inactivation of *DBC2*. We can now study consequences of *DBC2* ablation at the cellular level by using this system.

One of our approaches is a microarray analysis that allows us to monitor thousands of genes at the same time. The microarray we use is a microscope slide carrying 46,000 spots in a 1 x 2-inch area. Each spot contains a DNA probe representing a gene. When a color-labeled sample is incubated with the microarray, DNA fragments in the sample seek matching with the probe. As a result, the color intensity of a probe corresponds to the amount of the matching sequences in the sample. When cDNA from a specimen is used, the color intensity indicates the expression level of the gene represented by the probe. This is particularly useful when we want to isolate genes that are expressed differently in the two specimens. The microarray analysis has isolated 108 genes that are up-regulated by *DBC2* activation. Additionally, consequences of *DBC2* knockdown by RNAi are also investigated by this approach. We are currently studying how *DBC2* regulates the expression of these genes (directly or indirectly) and what role *DBC2* has in the biological pathways involving these genes.

Resources for *DBC2* Analysis

M. Hamaguchi, N. Kobayashi [in collaboration with Antibody Shared Resource, Cold Spring Harbor Laboratory]

Antibodies are fundamental materials to study a new protein. We have raised polyclonal and monoclonal antibodies against *DBC2*. These antibodies are used for immunobloting and immunoprecipitation, revealing valuable information.

We have generated various *DBC2* mutants for functional analysis. Some are a recreation of somatic mutations discovered in breast cancers. They are used to determine if the mutants are capable of suppressing tumor growth. Dysfunctional mutants are then tested for other biological functions including protein-protein binding and GTPase activity. Mutants lacking each functional domain were also generated. They are used to verify functions of the domain.

Expression of *DBC2*

M. Hamaguchi, J. Meth [in collaboration with D. Broek, University of Southern California]

Tumor suppressor genes are inactivated by transcriptional suppression in some cases. In fact, *DBC2* expression is extinguished in approximately half of breast and lung tumors, whereas its expression is detected in other types of cancer. Modification of the promoter region has been shown to cause transcriptional inactivation. The most common mechanism for such modification is methylation. A number of cancer cell lines derived from breast and lung cancers were found to possess hypermethylated promoter regions. Transcriptional inactivation by hypermethylation can be reversed in many cases when methylation is chemically blocked. A commonly used chemical for this purpose is 5-aza-2′-deoxycytidine (5-Aza-C). We cultured these cells with 5-Aza-C and demonstrated that *DBC2* expression was restored. Our findings suggest that methylation of the *DBC2* promoter is the main mechanism for silencing.

Analyzing Functional Domains of *DBC2*

M. Hamaguchi, N. Kobayashi, R. Shudo [in collaboration with L. Van Aelst, Cold Spring Harbor Laboratory]

Computational analysis revealed that *DBC2* contains putative protein-protein interaction domains (BTB). We utilized immunoprecipitation and a yeast two-hybrid system to isolate five candidate proteins. Many of them are known to participate in carcinogenic cellular pathways. We are currently trying to understand how *DBC2* is involved in these pathways in the hope that we may be able to discover good targets for new therapy.

Generation of *DBC2* Knockout Mice

M. Hamaguchi, J. Meth [in collaboration with the Genomic Targeting and Transgenic Mouse Facility, Cold Spring Harbor Laboratory, and M. Zhang, Cold Spring Harbor Laboratory]

Analysis of a mouse model has advantages. First, more information has been accumulated from mouse genetics than that of any other vertebrate. Second, gene functions can be studied in vivo. Analysis of tumor suppressor genes has often been facilitated by studies of tumor-suppressor deficient mice.

Mouse embryonic stem (ES) cells were genetically engineered so that they had a disrupted *DBC2* allele. These ES cells were used to produce chimeric mice that are partially originated from the ES cells. The chimeric mice were examined and successful introduction of the engineered *DBC2* allele was confirmed. We were able to generate heterozygous knockout mice from the chimeric mice. However, homozygous knockout mice were proven to be lethal, forcing us to seek alternative strategies such as long-term observation of heterozygous knockout mice, promoting carcinogenesis in heterozygous knockout mice, and generation of conditional knockout mice. We are continuing our efforts to establish mouse models for future research.

RNA INTERFERENCE: MECHANISMS AND APPLICATIONS

G. Hannon M. Carmell, A. Denli, J. Du, L. He, I. Hoffa, J. Liu, C. Marsden, L. Murchison, P.J. Paddison, F. Rivas, Y. Seger, J.M. Silva, D. Siolas

Several years ago, work in a free-living nematode, *Caenorhabditis elegans*, uncovered a previously unknown biological response through which an organism exposed to double-stranded RNA specifically silenced genes that share homology with that nucleic acid. This phenomenon, called RNA interference (RNAi), has since been shown to be an evolutionarily conserved pathway, present in organisms ranging from fungi to plants to mammals. We have striven to understand the mechanistic basis of this response using biochemical approaches in several systems, including *Drosophila* and mammalian cells. We have identified numerous components of this pathway, including the key initiating enzyme, Dicer and components of the RISC (RNA-induced silencing complex) effector machinery. These efforts continued last year, leading to the identification of a new element of the pathway, a micrococcal nuclease relative, TSN-1, which is a RISC component in *Drosophila*, *C. elegans*, and mammals. Two of the signature components of the pathway, Dicer and Argonaute proteins, share a common domain that is found only in these protein families, the PAZ domain. Last year, we worked with Leemor Joshua-Tor here at CSHL to understand this domain both structurally and biochemically. These studies led to the hypothesis that this domain functions to bind to the specific terminal structures of the small interfering RNAs (siRNAs) that are central to the RNAi response. Our efforts to understand the biological function of the RNAi machinery in mammals have begun to bear fruit with the creation of genetically modified mice that lack the Dicer enzyme. These mice die early in development and are characterized by a depletion of multipotent stem cells from the early embryos. This work shows for the first time that the integrity of the RNAi machinery is essential for mammalian development. As we continue to understand the mechanism and function of the RNAi machinery, we gain the opportunity to develop ways to exploit this response for experimentally manipulating gene expression in mammals. Last year began with our demonstration that we could use RNAi to generate heritable loss of function in mice. Parallel studies with Scott Lowe's lab here at CSHL showed that we could use RNAi in engineered stem cells to create mosaic animals in which those cells gave rise to genetically defined tumors in mice. Taking these experiments from the single-gene level to a point at which we could use RNAi to address the function of every gene in the human and mouse genome has been our long-term goal. Last year, with the help of Dick McCombie here at CSHL, Steve Elledge at Harvard, and colleagues at Merck (CSHL alumnus, Michele Cleary) and OSI (Julie Kan), we have developed a large-scale library of RNAi-inducing vectors that presently covers approximately one third of known human genes. We have validated this library in pilot screens and are now beginning to use this tool to search for new targets for cancer therapy. Examples of specific projects are elaborated in more detail, below.

Last year saw the departure of several valued colleagues. Emily Bernstein was granted her Ph.D. from Stony Brook University, New York, and Amy Caudy became the first graduate of the Watson School of Biological Sciences. Long-time associate Doug Conklin accepted a faculty position in Albany. We were visited during the year by Dave Kuppersmith, Hana Mizuno, Bas Tops, and Jonathan Schneiderman and were joined by a research associate, Ken Chang, postdocs Jidong Liu, Faby Rivas, and Lin He, and graduate students Liz Murchison and Despina Siolas.

Control of Centrosome Replication and Function

J. Du

During the last year, we continued our efforts to dissect the genetics of the role of the Aurora-A kinase in human tumorigenesis. Following 2002's work to purify the Aurora-A complex (SAF) from mammalian cells and characterize its components, we found p160ROCK to be a genetic suppressor of the inactivation of Aurora-A (Du and Hannon, submitted). We therefore focus now on astrin, an Aurora-A-interacting protein isolated by a two-hybrid screen. Astrin is a

mitotic-spindle-associated protein that is co-overexpressed with and localized together with Aurora-A temporally and spatially at the mitotic spindles from the beginning of prophase to anaphase. Astrin also associates with the kinetochore at mitosis, as shown by colocalization with CENP-E. Astrin coimmunoprecipitates not only with Aurora-A, but also with α-tubulin, a building block of mitotic spindles. Corresponding to the astrin expression level at mitosis, this association of astrin with α-tubulin is more prominent in mitotic cells, and its localization is sensitive to microtubule dissipation reagents, such as Nocodazole. Depletion of astrin by RNAi in HeLa cells showed a moderate G_2/M cell cycle delay, similar to the G_2/M arrest phenotype in siAurora-A-treated cells. This prolonged G_2/M delay eventually leads to apoptosis. Immunofluorescence examination showed that the G_2/M delay in siAstrin-treated cells is caused by mislocation of cyclin B1 to centrosome and mitotic spindles in mitotic cells, and subsequently the inactivation of CDK1 kinase activity. Astrin inactivation by RNAi induces the formation of multiple mitotic spindles when cells progress into prometaphase. Only two of the resulting γ-tubulin-positive spots contain centrin2, a marker of authentic centrosomes, suggesting that the other multipolar spindles irradiate from de novo reorganization and accumulation of preexisting r-tubulin. A kinesin Eg5 inhibitor, monastrol, suppresses the multipolar spindle phenotype, suggesting its formation in the absence of astrin requires Eg5 motor protein. Inactivation of astrin decreases Aurora-A localization at centrosome in interphase cells and depletes it from microtubule spindles, although it does not affect its centrosome localization and phosphorylation at Thr-288 in mitotic cells. The mitotic arrest phenotype in double depletion of Aurora-A and astrin plus the Aurora-A dislocation from the mitotic spindle in siAstrin-treated cells all suggest that astrin is a upstream regulator of Aurora-A. Overexpression of astrin causes senescence in primary human fibroblast IMR90 cells because it significantly elevates the p53 level in the cells.

Human Cell Transformation

Y. Seger

Our knowledge of the transformation process has emerged largely from studies of primary rodent cells and animal models. In primary rodent fibroblasts, the transformation process is well-defined and usually requires two genetic alterations. However, rodent transformation models do not perfectly recapitulate the transformation process in humans as it is impossible to transform normal human cells into tumor cells using the same oncogene combinations that are effective in rodent cells. One obvious difference between rodent and human cell models is the requirement for telomerase, the enzyme responsible for maintaining telomeres. In rodent cells, transformation is independent of exogenous telomerase induction due to promiscuous telomerase expression and constitutively long telomeres. The potential importance of telomerase in human transformation processes has been supported by the fact that a majority of human tumors are telomerase-positive. In addition, previous reports have indicated that the direct expression of the telomerase catalytic subunit hTERT is a vital component of the human cell transformation equation.

Our work over the past several years has resulted in the development of a human cell transformation system that is independent of direct induction of hTERT or a gene previously shown to be capable of activating telomerase. We have shown that the combined expression of adenovirus E1A, Ha-RasV12, and MDM2 (ERM) is sufficient for the conversion of a normal human cell into a cancer cell. Notably, these cells are telomerase-negative upon injection into nude mice, and the resultant tumors are also negative for telomerase activity. As a result, we see continuous telomere shortening and chromosomal abnormalities similar to those seen in telomerase-null mice. This suggests that alterations in telomere biology must be viewed similarly to genomic instability as catalysts of transformation, rather than central components of the transformed phenotype.

This ERM transformation model is now functioning as a highly tractable system within which several RNAi-based approaches can be applied. Most notably, we are currently utilizing stably expressed short hairpin RNA (shRNA) constructs to knock down expression of E1A targets in order to rescue functional deletion mutants of E1A and restore transforming function. Similarly, a hairpin rescue screen could eventually identify additional genes whose silencing contributes to E1A-mediated transformation.

Genetics of miRNA Function

L. He

microRNAs (miRNAs) are a family of 21–25-nucleotide small RNAs that negatively regulate gene

expression at the post-transcriptional level. To date, several hundred miRNAs have been identified in mammals, fish, flies, worms, and plants, about one hundredth of the protein encoding genes in each organism. Functional studies have only touched upon a handful of miRNAs—all in model organisms, which have an important role in regulating developmental timing and cell proliferation. To study the functions of mammalian miRNAs, I am constructing an expression library that directs ectopic synthesis of both the mouse miRNAs and the human miRNAs. This library will be used to search for oncogenic miRNAs using well-established transformation systems in vitro and will be used in vivo to search for roles of miRNAs in regulating development and differentiation, particularly in the hematopoietic compartment.

Building a Better Hairpin

D. Siolas

My work currently focuses on miRNA processing and enhancing applications of shRNA gene silencing in mammalian cells. Long primary (Pri)-miRNAs are processed intracellularly by the enzyme Drosha into shorter approximately 70-nucleotide pre-miRNAs that are hairpin structures with a 3′ two-nucleotide overhang. These premiRNAs are processed by an RNase III enzyme, Dicer, into mature 22-nucleotide miRNAs. In vitro studies using human recombinant Dicer have revealed that Dicer processes 29-mer shRNA into distinct 22-mer siRNA from its stem end in a single cleavage event, thus determining where an siRNA is produced in a given shRNA. A two-base 3′ overhang is necessary for efficient generation of 22-mer siRNA, and 22-mer processing is greatly reduced in hairpins with no overhang or with a 5′ overhang. In addition, hairpins of different lengths were examined, and although 29-mer hairpins were processed efficiently into 22-mers, hairpins of 21 nucleotides were processed inefficiently, resulting in products of varying sizes; 19-mer hairpins were not processed. These insights allowed us to devise methods to design specific shRNAs that would produce predictable siRNAs in vivo. In collaboration with Michelle Cleary (CSHL alumnus now at Rosetta Inpharmatics), we tested the ability of algorithms that predict effective siRNAs to predict similarly effective shRNAs. Strikingly, when siRNAs are provided to the cell in the context of a 29-nucleotide shRNA, it is more effective than if delivered directly as an siRNA, with shRNAs showing not only more potent inhibition (better silencing per nanogram delivered to cells), but also more complete endpoint inhibition. These studies have permitted us to develop a new type of more effective RNA-based trigger of RNAi and also to design the next generation of genome-wide RNAi libraries.

RNAi and the Genome

A. Denli

RNA has recently become the centerpiece of gene silencing after the discovery that double-stranded RNAs trigger gene silencing through different effector complexes. Even though the discovery of this phenomenon (RNAi) has been through using exogenous dsRNA, further studies revealed that cells use RNAi for endogenous gene regulation and even heterochromatin formation. My interest has been the mechanism of these endogenous processes. Data from my work suggest that the RNAi machinery is biochemically conserved in the fission yeast *Schizosaccharomyces pombe*, and some novel interactions observed between RNAi proteins may help bring the blurry picture of RNAi-heterochromatin interplay into better focus. My other project involves miRNA biogenesis, in which I am trying to purify complexes involved in primary-miRNA processing. I believe my studies, and the work of my colleagues in the lab and elsewhere, will allow us to better characterize the RNAi pathway, to reveal its connections to other phenomena, and to use RNAi as an even better tool to silence genes in numerous organisms, most importantly mammals.

Biological Functions of RNAi in Mammals

M. Carmell

Argonaute (Ago) family members have been shown to be essential for RNAi/PTGS (post-translational gene silencing) in several organisms, including *Neurospora* (QDE-2), *Arabidopsis* (AGO1), and *C. elegans* (*rde-1*). Argonautes are core components of RISC complexes, and function both in mRNA destruction and translational inhibition. Interestingly, Argonaute genes have also been implicated in control of development in several organisms. It is unknown what roles of the RISC complex are mediated by Argonaute family

members, and whether developmental phenotypes result from loss of discrete regulatory functions or from a more general misregulation of silencing mechanisms. To address the role of Argonautes in the mammalian system, we have undertaken a comprehensive analysis of mouse mutants engineered to lack individual Argonaute family proteins. Thus far, three of seven mammalian Ago genes have been disrupted, and informative phenotypes are beginning to emerge.

The *Ago2* mutant embryos display severe developmental defects and survive only halfway through gestation. The phenotype includes a neural tube closure defect and a probable heart defect. Current studies are examining whether these phenotypes are due to perturbation of embryonic patterning pathways or to genome-wide silencing issues.

Embryos homozygous for *Miwi2*, which is an Argonaute gene primarily expressed in the testes, exhibit defects in spermatogenesis. *Miwi2*-deficient males are sterile, as the testes lack all postmeiotic cell types. Studies of *Ago1* and *Ago3* are also under way.

RNAi in Mammals: The Quest for Illuminating Gene Function

P.J. Paddison, J.M. Silva

Since the 1970s, the war on cancer has been based on the notion that studying the disease will lead to the discovery of vulnerabilities, which can be exploited in the clinic. Many underlying genetic determinants of cancer have been identified, but this knowledge has failed to translate into new therapeutic strategies, with only a handful of exceptions. This is largely due to the genetically intractable nature of cultured mammalian cells.

The immergence of RNAi as a technology to silence gene expression in virtually every experimental eukaryotic system, including mammals, holds promise for identifying cancer lethal targets. Our work has revolved around (1) demonstrating that double-stranded RNA induces sequence-specific gene silencing in mammalian cells (Paddison et al., *Proc. Natl. Acad. Sci. 99:* 1443 [2002a]); (2) refining the method of triggering RNAi so that it can be applied in most cellular contexts (Paddison et al., *Genes Dev. 16:* 948 [2002b]; Paddison and Hannon *Cancer Cell 2:* 17 [2002]; Hemann et al. 2003); and (3) constructing genome-wide RNAi libraries to make genetic screens feasible in mammals (Paddison et al. 2004).

During the past year, we have constructed a mammalian RNAi library targeting approximately 12,000 human genes and about 8,000 mouse genes. This library is presently composed of about 30,000 sequence-verified shRNA expression cassettes contained within multifunctional vectors, which permit shRNA cassettes to be packaged in retroviruses, tracked in mixed cell populations via DNA "bar codes," and shuttled to customized vectors by bacterial mating. For library validation, we employed a genetic screen designed to report defects in human proteasome function. Our results suggest that our large-scale RNAi library can be used in specific genetic applications in mammals and will likely become a valuable resource for gene analysis and discovery.

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Michelle Carmell

SPORADIC HUMAN GENETIC DISEASE

E. Hatchwell S. Kantarci S. Mehra
T. Lee P. Slomiany

We are interested in the application of modern methodologies to the analysis of sporadic human genetic disorders. Many disorders are now known to be associated with de novo mutations; this knowledge has been gleaned almost exclusively as a result of serendipitous observations of cytogenetic abnormalities in unique individuals with the relevant disease. It is no surprise that many disorders are associated almost exclusively with de novo mutations as affected individuals do not reproduce (for a variety of reasons). The technology to detect de novo point mutations is some way off (i.e., this would require whole-genome sequencing in a rapid and cost-effective manner), but we now have tools to detect a class of mutations that are known to be relevant to this group of disorders, namely, copy-number abnormalities. The mainstay of whole-genome analysis historically has been chromosome analysis, but this suffers from a lack of resolution (abnormalities smaller than 10 Mb are not routinely detectable).

Genome-wide copy-number array analysis at high resolution is now available using a number of different formats, including large clone, cDNA, and oligo arrays. Our preference is for large-clone genomic arrays, using material amplified from well-characterized bacterial artificial chromosomes (BACs), because these have been shown to be both robust and capable of detecting very subtle copy-number abnormalities, including 3:2 (such as result from heterozygous duplications).

During the last year, we have consolidated our use of BAC arrays in a variety of disease groups and have also initiated relatively novel methodologies for the validation of array detected copy number abnormalities. We continue to be interested in a variety of disease groups:

- Congenital heart disease (CHD) in collaboration with pediatric cardiologists both in the United States and the United Kingdom to search for copy-number variations in children with CHD who are 22q11 normal (22q11 deletions are the most common currently known cause of CHD).
- Complex phenotypes, mostly involving mental retardation in association with physical anomalies, in individuals with normal karyotypes.
- Individuals with sporadic phenotypes in association with de novo balanced translocations, as a collaboration with the Mendelian Cytogenetics Network (MCN).
- Autistic spectrum disorder, in collaboration with workers at the Cody Center, Stony Brook University, New York.
- Essential thrombocytosis (ET) in collaboration with workers at Stony Brook University. We shall be performing genomic analysis in individuals with ET. It is known that many ET patients have large deletions of 5q, and we are planning to both map these more precisely and also uncover smaller deletions in patients without obvious cytogenetic abnormalities.

Genome-wide BAC Arrays

T. Lee

We have generated BAC arrays using a combination of available protocols and universal amplification methods that we have developed in-house. Our initial arrays were based on two distinct sets of BACs isolated by two independent groups:

1. The Cheung laboratory (http://genomics.med.upenn.edu/genmapdb/). This set consists of approximately 4200 BACs isolated by STS screening of regularly spaced markers in the genome. Most of these BACs were not FISH-mapped. These arrays (with an average resolution of <1 Mb) have been instrumental in detecting a number of interesting copy-number abnormalities in our patient groups (see below).
2. The Cancer Chromosome Aberration Project (CCAP; http://cgap.nci.nih.gov/Chromosomes/CCAP_BAC_Clones). This set consists of approximately 1300 FISH-mapped BACs selected on the basis that they had been either completely sequenced or anchored to the human genome by virtue of end-sequence information. The resolution of these arrays is relatively low, but their utility lies

in the knowledge that all elements recognize a united genomic address.

This year, we have generated 27,000 element BAC arrays (~9000 BACs arrayed in triplicate) that combine the two sets described above and also the set from the Sanger Centre, Cambridge, UK, which contains ~3000 BACs which have been fingerprinted. Our current arrays therefore have an average resolution of 300 kb and will be used in 2004 for analysis of the patient groups described above.

Characterization of a Novel Gene in Joubert Syndome

S. Kantarci

We have previously mapped the chromosome 2q13 breakpoint in an individual with a de novo balanced translocation t(2;22)(q13;q11.1) associated with Joubert Syndrome (JS) (OMIM 213300). JS is a rare disorder that is associated with mental retardation, hypoplasia of the cerebellar vermis, respiratory abnormalities, and occasional renal and retinal anomalies. Genes for this disorder have not yet been isolated. Using BAC array analysis of flow-sorted chromosomes , we were able to rapidly map the breakpoint on chromosome 2 and have recently isolated a novel 10-exon gene, which spans more than 500 kb of the genome. The translocation directly interrupts this gene (between exons 1 and 2) and is currently being considered as a candidate for JS in those patients with no obvious cytogenetic abnormality, in whom linkage has not excluded 2q13.

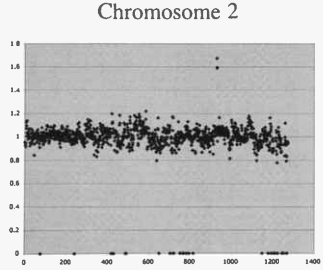

FIGURE 1 Output for chromosome 2 in a patient with a complex phenotype (see text). The 3 spots with an elevated ratio are from the same BAC, which was subsequently shown to be deleted (ratios are Cy5/Cy3, the patient was labeled with Cy3 and the control with Cy5).

Characterization of a De Novo Microdeletion at 2Q31

P. Slomiany

Using our genome-wide BAC arrays, we have detected a submicroscopic deletion in a patient with a phenotype reminiscent of velocardiofacial syndrome (VCF, associated in the majority of cases with a heterozygous microdeletion at 22q11). This patient presented with microcephaly, developmental delay, and short stature, as well as dysmorphic facies. Routine cytogenetic analysis was reported as normal. The microdeletion detected is at 2q31, and we have now shown that this microdeletion is de novo, making it extremely likely that the deletion is causative in this individual (his parents are phenotypically normal and neither is deleted in this region). The initial detection of the deletion was made using BAC arrays with an average resolution of 1 Mb (based on the Cheung set). We subsequently generated a tiling path array using 40 BACs from chromosome 2q31, in an attempt to narrow down the deletion end points. This region-specific array was generated using a personal microarrayer: the SpotBot from Telechem. The SpotBot is a 1-foot cube arrayer, which is controlled by software from a laptop and which can generate up to 12 arrays in about 2 hours. It is ideal for region-specific arrays and for testing out novel array chemistries/probe types.

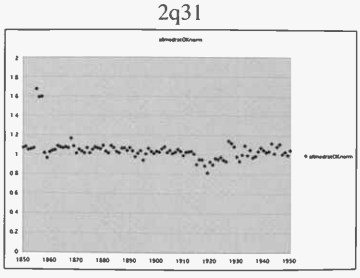

FIGURE 2 Closer view of the chromosome 2 output shown in Figure 1.

Our region-specific microarrays have allowed us to determine the endpoints of the deletion to within 100 kb. Furthermore, we have isolated the deleted chromosome 2 from our patient after fusion of lymphoblastoid cells with hamster cells and analysis of hybrid clones using STRs from chromosome 2. We are now in a position to completely map the deletion and determine which genes are contained therein, in order to try and draw phenotype-genotype correlations.

It is likely that a 2q31 microdeletion exists in other individuals with similar phenotypes, and we are currently analyzing 22q11 negative VCF look-alikes for the presence of this deletion. We fully expect to be able to delineate a new 2q31 microdeletion syndrome once our studies are complete.

Validation of Array-detected Copy-number Abnormalities

S. Mehra

Although it is clear that array analysis has revolutionized much of molecular biology, little attention has been given to the validation of ratiometric changes detected by array analysis, whether using genomic or expression arrays. The mainstay of validation of copy-number abnormalities is quantitative (real-time) polymerase chain reaction (PCR), but this method is inadequate for subtle changes (namely, 2:1 or less). Few alternatives exist to address this problem:

2q31 tiling path array

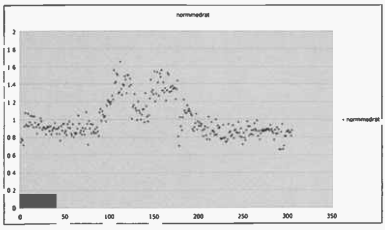

FIGURE 3 Tiling path array of the 2q31 region implicated in a deletion in our patient. This array has an approximate resolution of 100 kb and has allowed us to delineate more precisely the endpoints of the deletion. The "dip" in the middle is likely due to homology with another region in the genome.

1. FISH (fluorescence in situ hybridization) analysis is useful for confirming heterozygous deletions (2:1) and (usually) heterozygous duplications (3:2) but suffers from a number of drawbacks, namely, the necessity for cells and the limitations in confirming small heterozygous duplications, which often appear as a single "spot," rather than two distinct signals. Since FISH is not quantitative, it is impossible to differentiate between the brighter signal obtained when a small tandem duplication is present from a normal signal.
2. Dosage analysis using Southern blots is labor-intensive, requires large amounts of material, and very accurate determinations of DNA concentration.
3. STR (short tandem repeat) analysis may be useful but cannot formally differentiate between homo- and hemizygosity when attempting to validate deletions. Furthermore, useful STRs may not be available for very short regions. Finally, when attempting to confirm heterozygous duplications, STRs are only useful if three distinct alleles are observed (unlikely to be present routinely), or if suitable methods are available for dosage analysis of the two distinct bands (when the STR is informative).

We have determined that the most effective methods for validating suspected copy-number changes involve "counting" single molecules from regions of interest and from control regions. Theoretically, this approach should be able to validate very subtle changes (say <10%), such as occur in mosaic states. A number of methodologies exist for single molecule counting, but we have chosen polony technology, as we believe this to be the most practical. The basic principle is simple: Limiting dilutions of genomic DNA are added to a polyacrylamide matrix containing PCR reagents on a slide, and the slides are then subjected to thermal cycling. Amplification of the cognate sequences on single molecules results in PCR colonies (i.e., polonies), which can then be detected using fluorescently labeled DNA probes. If this is done in parallel on test and control samples, using control primers and primers from the region of interest, it should be possible to obtain very accurate ratios of the number of molecules represented, respectively, by the two regions being compared. For example, a mosaic deletion analyzed on an array may be associated with a ratio of about 0.8, which might reasonably be considered close enough to 1 to ignore, whereas the ratio obtained from a polony experiment would be, say about 80/100, where these numbers correspond to the number of molecules amplified.

REGULATION OF APOPTOSIS AND NUTRIENT METABOLISM IN CANCER CELLS

Y. Lazebnik C. Cormier A. Matapurkar
D. Duelli X. Opitz-Araya
S.-C. Lin M. Yuneva

Treatment of cancer relies on differences between normal and cancer cells that allow for selective or preferential killing of cancer cells. In a search for these differences, we have been focusing on apoptosis, and recently, on the requirement for nutrients.

Apoptosis is a type of cell death that is carried out by specialized cellular machinery. The current knowledge is sufficient to design and implement tools that kill cells quickly and efficiently by inducing apoptosis. However, the major problem is how to induce apoptosis in cancer cells selectively. One approach to solving this problem is to learn how apoptosis is induced by oncogenic transformation. This approach is based on a paradoxical observation that some oncogenes, including *myc* and adenovirus E1A, either induce apoptosis or sensitize cells to cytotoxic agents, including those used for chemotherapy. One implication of this observation is that some oncoproteins exhibit proapoptotic activities specific for transformed cells. If true, then understanding how the apoptotic machinery is regulated by oncogenes may help to develop ways to kill cancer cells selectively.

Caspases are proteases that are activated at the onset of apoptosis and cause death by cleaving a number of proteins in a coordinated manner. Caspase activation occurs in two steps. At the first step, pro-apoptotic signals lead to autocatalytic activation of caspases that are called initiators. Activated initiator caspases process effector caspases, which in turn cause cell collapse by cleaving a specific set of substrates. Each initiator caspase is activated in response to a subset of signals, indicating that a prerequisite for understanding how a specific signal activates apoptosis is finding the initiator caspase that mediates it. We have been investigating which caspases are involved in apoptosis and studying how they are activated, how this activation leads to cell death, and what prevents this activation in drug-resistant cells. The ultimate goal is to understand how caspases can be activated selectively in cancer cells.

To investigate how caspases are regulated by oncogenes, we previously developed a cell-free system that mimics apoptosis dependent on expression of *E1A*, an adenoviral oncogene. Using this system, we found that *E1A*-dependent apoptosis is mediated by caspase-9 and that the expression of *E1A* sensitizes cells to apoptosis by facilitatating activation of this caspase. This is achieved by at least two ways: facilitating the release from mitochondria of cytochrome *c*, a cofactor required for caspase-9 activation, and regulation of an unidentified subsequent step in caspase-9 processing.

This model was consistent with the prevailing view that cytotoxic stress, such as DNA damage, induces apoptosis by regulating permeability of mitochondria. Mitochondria sequester several proteins that, if released, kill by activating caspases, the proteases that disassemble the cell. Another way to activate caspases, which is used by cytokines, is to assemble receptor complexes that activate caspases directly, although the subsequent mitochondrial permeabilization accelerates cell disassembly by amplifying caspase activity. We found that cytotoxic stress causes activation of caspase-2 and that silencing expression of this caspase with a small interfering RNA (siRNA) prevents permeabilization of mitochondria and, subsequently, apoptosis. Therefore, we concluded that cytokine and stress-induced apoptosis act through conceptually similar pathways in which mitochondria are amplifiers of caspase activity, rather than initiators of caspase activation.

During the last year, however, we found that silencing caspase-2 with a different siRNA has no effect on apoptosis. This result questioned our previous conclusion and suggested three possibilities: (1) These siRNAs differentially silence caspase-2 isoforms, which alters the outcome of drug-induced apoptosis; (2) one of the two siRNAs silences an unidentified gene(s), whose product is involved in apoptosis; and (3) one of the two siRNAs has some effect unrelated to RNAi. We have been investigating all these possibilities, but have yet to determine which of them is correct.

Our studies of the link between apoptosis and

oncogenes have recently led us to focus on the changes that these oncogenes make in cell metabolism, and how these changes affect cell viability. These studies are just beginning to provide interesting results that we hope may open new opportunities for manipulating cell viability to selectively kill cancer cells.

During this year, we have also continued to expand our observation, which is consistent with a body of largely forgotten literature, that cell fusion is linked to cancer. We have summarized existing arguments in favor of this idea and reported some of our hypotheses in an opinion letter that argues that this link should not be ignored without investigating it. We also continued our collaborations with several laboratories. Overall, our studies continued to investigate how to kill cancer cells selectively.

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Anagha Matapurkar (*left*), graduate student, and Maria Yuneva, postdoc, in the Lazebnik lab.

REGULATION OF APOPTOSIS AND SENESCENCE BY CANCER GENES

S. Lowe A. Bric, D. Burgess, E. Cepero, E. de Stanchina, R. Dickins, M. Hemann, M. McCurrach, M. Narita, S. Nuñez, S. Ray, C. Rosenthal, C. Scott, M. Spector, H. Wendel, M. Yang, J. Zilfou

Apoptosis is a genetically controlled form of cell death that is important for normal development and tissue homeostasis. Senescence produces "genetic death" in that the senescent cell is incapable of further propagation. Both processes are frequently disrupted in cancer cells, implying that each can limit tumor development. Moreover, radiation and many chemotherapeutic agents can induce either apoptosis or senescence, raising the possibility that the integrity of these programmed responses influences the outcome of cancer therapy in patients. The goal of our research is to understand how cancer genes control apoptosis and senescence in normal cells, and how mutations that disrupt these processes impact tumor development and therapy. Our approach emphasizes genetics, and we typically exploit simple cellular systems to study cancer gene function. We also study animal models and human tumors to confirm the relevance of our simple systems for tumor development and cancer therapy in vivo.

Control of Apoptosis by Oncogenes and Tumor Suppressor Genes

A. Bric, D. Burgess, E. Cepero, E. de Stanchina, M. Hemann, M. McCurrach, J. Zilfou [in collaboration with C. Cordon-Cardo and P. Pandolfi, Memorial Sloan-Kettering Cancer Center, New York; also with former laboratory member J. Fridman]

Normal cells possess natural fail-safe mechanisms that limit the consequences of aberrant proliferation. For example, deregulated expression of c-Myc or disruption of the Rb (retinoblastoma) pathway in normal cells can force aberrant S-phase entry and predispose cells to apoptotic cell death. This increased sensitivity to apoptosis acts in part via the p53 tumor suppressor pathway and limits tumor development. We have previously shown that oncogenes can engage the ARF-p53 pathway to promote apoptosis, and that disruption of this pathway cooperates with oncogenes to promote oncogenic transformation in vitro and tumor development in vivo (for review, see Lowe and Sherr 2003). We are currently interested in identifying additional components of these programs and understanding how they function in a "tumor suppressor network."

p53 acts via transcription-dependent and -independent mechanisms that act in concert to target multiple levels in order to promote efficient cell death (Fridman and Lowe 2003). This year, we identified the promyelocytic leukemia (PML) gene product as a new factor that modulates p53-dependent apoptosis (de Stanchina et al. 2004). Specifically, we showed that Myc-expressing cells lacking PML are defective in apoptosis, albeit not as defective as p53-null cells. Interestingly, PML appears to modulate p53-dependent apoptosis, at least in part, by acting as a direct p53 effector (see below). PML, in turn, cooperates with other p53 effectors to promote efficient cell death. How this occurs remains to be determined and is a topic of current research.

The Mdm2 oncoprotein physically associates with p53 and antagonizes its tumor suppressor functions. Interestingly, some human and murine tumors express high levels of alternatively or aberrantly spliced Mdm2 variants that are unable to bind p53, but whether these actively contribute to carcinogenesis or are a byproduct of cancer progression has been unclear. This year, we examined the ability of full-length Mdm2 and several tumor-derived splice variants to modulate tumor development in *Eμ-myc* transgenic mice (Fridman et al. 2003). We showed that several tumor-derived Mdm2 splice variants promote tumorigenesis in a manner that is comparable to that of full-length Mdm2. Our results imply that the current paradigm for understanding Mdm2 action during oncogenesis is incomplete and that human Mdm2 splice variants can contribute to human cancer.

We also are involved in several collaborative studies with CSHL scientists. For example, with William Tansey, we are dissecting the function of the Myc oncoprotein using mouse models of leukemia and lymphoma and are comparing and contrasting the mechanisms by which the E1A and Myc oncoproteins promote apoptosis. With Gregory Hannon, we are using libraries of murine "short hairpin" RNAs to dissect the apoptotic network in cultured cells and in animals (see below). These studies have enormous potential to produce new insights into p53 function and the control of apoptosis over the next several years.

Control of Cellular Senescence

E. de Stanchina, M. Narita, M. Narita, S. Nuñez, J. Zilfou [in collaboration with G. Hannon; also involving former laboratory members E. Querido and G. Ferbeyre]

Cellular senescence was originally described as the process that accompanies replicative exhaustion in cultured human fibroblasts and is characterized by a series of poorly understood markers. Senescent cells remain metabolically active, but they are unable to proliferate and display changes in gene expression that could alter tissue physiology. As such, they are genetically "dead" and cannot contribute to tumor development. Although "replicative" senescence is triggered by telomere attrition and can be prevented by telomerase, an identical endpoint can be produced acutely in response to activated oncogenes, DNA damage, oxidative stress, and sub-optimal cell culture conditions. These observations have led us to propose that senescence acts in parallel to apoptosis as a cellular response to stress. Indeed, based on this analogy, much of our work on senescence is guided by our past experience on apoptosis.

One focus of our efforts is to identify new components that regulate senescence. We previously used microarray technology to identify the PML gene which is up-regulated during senescence. Until recently, most studies suggested that PML acts upstream of *p53* to enhance its transcription of targets by recruiting *p53* to nuclear bodies (NBs). However, this year, we show that PML is a *p53* target gene that also acts downstream from *p53* to potentiate its antiproliferative effects (de Stanchina et al. 2004). Hence, *p53* is required for the induction of PML and NBs in response to oncogenes and DNA damaging chemotherapeutics. Furthermore, the PML gene contains consensus *p53*-binding sites that confer p53 responsiveness to a heterologous reporter and are capable of binding *p53* in vitro and in vivo. Finally, cells lacking PML show a reduced propensity to undergo cellular senescence or apoptosis in response to *p53* activation, despite the efficient induction of several *p53* target genes. These results identify a new element of PML regulation and establish PML as a mediator of *p53* tumor suppressor functions.

We are also actively studying the effector mechanisms involved in executing cellular senescence. This year, we described a distinct heterochromatic structure that accumulates in senescent human fibroblasts, which we designated senescence-associated heterochromatic foci (SAHF) (Narita et al. 2003). SAHF formation coincides with the recruitment of heterochromatin proteins and the Rb tumor suppressor to E2F-responsive promoters and is associated with the stable repression of E2F target genes that normally promote proliferation. Notably, both SAHF formation and the silencing of E2F target genes depend on the integrity of the Rb pathway and do not occur in quiescent cells that are capable of resuming proliferation with an appropriate stimulus. Therefore, our results provide a molecular explanation for the stability of the senescent state, as well as new insights into the action of Rb as a tumor suppressor (for review, see Narita and Lowe 2004]. We are currently expanding on these results in an effort to gain new insights into this potent tumor suppressive mechanism.

New Mouse Models of Cancer

E. Cepero, R. Dickins, M. Hemann, S. Ray, C. Rosenthal, H. Wendel, M. Yang, J. Zilfou [in collaboration with G. Hannon, Cold Spring Harbor Laboratory and C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center; also with former laboratory member J. Fridman]

During the last several years, we have developed rapid methods for producing transgenic and "knockout" animals as an alternative to germ-line methods. In one approach, we introduce genes into stem cells and then reconstitute the hematopoietic compartment of lethally irradiated mice. As a consequence, normal mice are produced that contain a "transgenic" hematopoietic

system. Both the transgene and the genotype of the stem cells can be varied, allowing one to rapidly produce chimeric animals with multiple genetic changes at much more rapid rates than would be possible with traditional germ-line methodology. Moreover, since our retroviral vectors often co-express a green fluorescence protein (GFP) reporter, it is possible to track the infected cells in vivo by whole-body fluorescence imaging. We have used this system to study the action of various oncogenes and tumor suppressor genes on the development and treatment of leukemia and lymphoma.

In collaboration with G. Hannon, we investigated whether RNA interference (RNAi) technology could be used to suppress gene expression in stem cells and produce phenotypes in reconstituted mice (Hemann et al. 2003). As a proof-of-concept experiment, we asked whether short hairpin RNAs (shRNAs) against *p53* could recapitulate the phenotype of complete *p53* deficiency during Myc-induced lymphomagenesis. We introduced several *p53* shRNAs into hematopoietic stem cells derived from *Eμ-myc* mice and monitored tumor onset and overall pathology in lethally irradiated recipients. Different *p53* shRNAs produced distinct phenotypes in vivo, ranging from benign lymphoid hyperplasias to highly disseminated lymphomas that paralleled the nullizygous setting. In all cases, the severity and type of disease correlated with the extent to which specific shRNAs inhibited *p53* activity. Therefore, RNAi can stably suppress gene expression in stem cells and reconstituted organs derived from those cells. Moreover, intrinsic differences between individual shRNA expression vectors targeting the same gene can be used to create an "epi-allelic series" for dissecting gene function in vivo.

Based on the success of these "proof-of-concept" studies, we are conducting a number of RNAi-based projects to dissect tumor phenotypes. One set of these involves experiments aimed at disrupting specific components of tumor suppressor networks and then examining how this affects tumor onset, pathology, and resistance to various therapies (see below). In another approach, we are collaborating with the Hannon laboratory to develop a library of murine "short hairpin" RNAs and have begun to use these in "forward genetic" screens for genes that influence tumor behavior. As our system is tractable, we hope to make rapid progress in this area. Finally, in addition to lymphoid models of malignancy, we are beginning to use these methods to model other cancers including acute and chronic myelogenous leukemias.

Molecular Genetics of Drug Sensitivity and Resistance

E. Cepero, M. Hemann, S. Ray, C. Rosenthal, A., C. Scott, M. Spector, H. Wendel, M. Yang, J. Zilfou [in collaboration with C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center; J. Pelletier, McGill University; S. Kogan, University of California, San Francisco; also with former laboratory member J. Fridman]

A major goal of our research is to understand the biological and molecular basis of drug sensitivity and resistance in tumors (e.g., see Soengas and Lowe 2003). Conventional approaches to identify factors that dictate treatment sensitivity rely on human tumor cell lines treated in vitro or as ectopic xenografts. As an alternative approach, we are using transgenic mouse models to study drug action in spontaneous tumors. Our system exploits the *Eμ-myc* transgenic mouse, which develops B-cell lymphomas at short latency with high penetrance. Using this system, we have identified a number of biologic and genetic determinants of treatment sensitivity in vivo.

In addition to characterizing drug resistance mechanisms, our most important goal is to use information from our models to develop and test better strategies to treat cancer. This year, we had our first major success in this effort, providing evidence that reversing apoptotic defects acquired during tumorigenesis could also overcome drug resistance (Wendel et al. 2004). Specifically, using the *Eμ-myc* model, we showed that the *Akt* oncogene accelerates tumorigenesis and promotes drug resistance in a manner comparable to the strictly anti-apoptotic protein Bcl-2. Remarkably, *Akt*-mediated drug resistance is reversed by the mTOR inhibitor rapamycin, which induces massive apoptosis and sustained responses, but only when combined with conventional chemotherapy. In contrast, rapamycin was unable to reverse drug resistance in Bcl-2-expressing lymphomas, despite their similar pathology to those expressing *Akt*. These results establish *Akt*-mediated apoptotic defects as an important mechanism of oncogenesis and drug resistance in vivo and reveal how targeting survival pathways can restore drug sensitivity in a genotype-dependent manner. Clearly, it will be important to determine whether similar rules apply in cancer patients.

Current efforts are focused at further dissecting *Akt* signaling and how it influences tumor development, drug resistance, and rapamycin sensitivity. In this regard, we have enlisted Dr. Jerry Pelletier

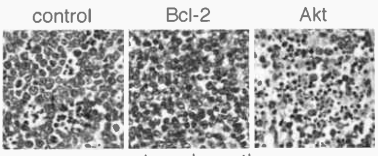

Figure 1 Tailoring chemotherapy based on cancer genetics. Mice harboring lymphomas of the indicated genetic background were treated with the combination of rapamycin and chemotherapy. Lymph nodes were isolated 6 hours later and lymphoma sections were stained with hematoxylin/eosin. This drug combination induces massive apoptosis in lymphoma expressing *Akt* but not those overexpressing Bcl2 or the controls, despite an overall similar pathology.

(McGill University) as a collaborator. We also are testing a number of novel "targeted" therapies in the *Eμ-myc* model in order to determine whether any can reverse or circumvent the drug resistance produced by *p53* loss. Finally, we have initiated a multi-institutional program (also involving scientists at UCSF, U. Minn., and U. Chicago) aimed at understanding and overcoming drug resistance in acute myelogenous leukemia (AML). Our program uses mouse AML models to uncover drug resistance mechanisms and to evaluate new therapies, with the goal of translating this information to human patients. We are excited about the potential of the SCOR, as it provides all of the elements necessary to validate the use of new mouse models as preclinical test systems.

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GENOMIC MICROARRAY ANALYSIS OF CANCER

R. Lucito A. Brady C. Hutter
S. Chen O. Dovirak

This work was carried out in collaboration with M. Wigler, J. Healy, A. Reiner, B. Mishra, and R. McCombie here at CSHL. Mutation of the genome is central to the development and progression of cancer. Mutations occur in the genome of the precancerous cells and accumulate, altering gene function until the growth of these cells goes unchecked. The genes responsible for cancer must be identified if we are to understand the cellular pathways that get subverted to allow the cell to become cancerous. We have begun surveying the genome of several cancer types to identify regions that have undergone increased or decreased gene copy number, namely, amplifications or deletions, since these changes can be used as markers for the location of oncogenes or tumor suppressor genes, respectively. Regions are being informatically searched for gene candidates, and then functional analysis of these candidates is being carried out to determine which genes have oncogenic potential. Once identified, these genes functions can be investigated to understand their role in the path to tumorigenesis. In addition by analyzing the regions alongside clinical data, we will determine if there are any regions or combination of regions that correlate to clinical outcome.

We have developed a genomic microarray technique, called representational oligonucleotide microarray analysis (ROMA), to identify copy-number fluctuations, which borrows the methodology of complexity reducing representations developed for RDA (representational difference analysis) to increase hybridization efficiency and increase signal to noise. A representation is a reproducible sampling of the genome, produced by first cleaving the genome with a restriction enzyme such as *Bgl*II, ligation of adaptors, and polymerase chain reaction (PCR) amplification. *Bgl*II representations of tumor and normal that are differentially labeled are compared on such an array to identify copy-number fluctuations. The array is composed of oligonucleotides based on the sequence of the human genome. We are using microarrays photochemically synthesized by Nimblegen Systems Inc. Currently, we are using arrays with 85,000 probes distributed throughout the genome and soon will be moving to an array of 191,000 probes.

The application of this type of an array spans many areas of biology, from cancer to genetic diseases. We will be using this microarray method to categorize the mutations that occur in primarily two tumor types initially, pancreatic and ovarian cancer.

PANCREATIC CANCER

There will be an estimated 30,000 cases of pancreatic cancer this year. Of these, 29,700 patients will succumb to the disease. Although the number of cases is low, in comparison to several other cancers such as colon, lung, or breast cancer, the survival rate for pancreatic cancer is one of the lowest. Treatments can extend survival or alleviate pain but seldom cure the patient. In fact, the mean survival time is approximately 6 months. Because the lifespan after diagnosis is very short, the number of patients that receive tumor resections is very low. This translates to few samples available for analysis. Because of this, we are collaborating with many clinicians to put together a useful tumor bank of pancreatic tissue for analysis by ROMA. We are currently collaborating with Dr. Ralph Hruban of Johns Hopkins, Dr. Daniel Von Hoff of the Arizona Cancer Center, Dr. Vijay Yajnic of Massachusetts General Hospital, and Dr. Murray Abrams of Memorial Sloan-Kettering Cancer Center, who will be providing pancreatic specimens and invaluable clinical information and expertise. We will be analyzing as many as 200 pancreatic tumor specimens. Due to several other issues involving pancreatic tumor specimens, approximately 25% of the samples will be xenograft samples or tumor specimens that have been grown in mice to passage then to a size that can be studied. ROMA will be performed for this tumor set to identify regions of the genome that have undergone copy-number fluctuations. We have analyzed close to 30 samples already and have confirmed the fact that the INK4a-ARF locus, DPC4, p53, and c-*myc* are all lesions commonly identified in this type of tumor. In addition, there are other less-characterized regions deleted (2p23, 3p, 8p23, 10p15, and 12p) and amplified (1q12, 1q42-43, 2q32, 3q26, 8q, and 12q) frequently in this cancer, and we are continuing to study these regions to identify possible gene candidate tumor suppressors and oncogenes. Eventually, the results from this study will be combined with clinical information associated with the patient to determine if there are any parameters that associate with specific tumor mutation patterns.

We are also focusing on the analysis of ovarian cancer using ROMA. Ovarian cancer has a relatively high incidence and approximately 50% survival rate. In many patients, the cancer is diagnosed late, often having metastasized, the first symptoms being an accumulation of fluid in the abdominal cavity. There have been few genes discovered that are involved in the progression of ovarian cancer. We will be collaborating with Dr. Michael Pearl of Stony Brook University, New York, for access to tissue and clinical information. We will also be performing ROMA on a tumor set of approximately 200 ovarian cancer samples to identify gene-copy-number fluctuations to identify candidate tumor suppressor and oncogenes. Ovarian cancer samples are more readily obtainable, and it is likely that all 200 samples analyzed will be primary tumor samples. We will be comparing samples of the primary cancer, tissue metastatic from the cell wall, tissue metastatic found in the fluid in the abdominal cavity, and in some cases, the metastatic tissue after chemotherapy. We intend to analyze the tumors to trace the accumulation of mutations from primary to metastasis with the hope of identifying not only genes involved in the development of the primary tumor, but also those genes that may increase metastatic capability and genes involved in chemotherapy resistance.

At present, we have analyzed 50 tumors and have compiled the data to identify regions commonly amplified or deleted. We have identified uncharacterized regions commonly amplified (1q21, 3q26, 6p, 11q13, 12p, and 20p13) and regions that are deleted frequently (1p35, 3p26, 4p15, 4q34, 5q14, 5q34, 6q22, 9q22, 12q, 13q13, 16q, 19p, and 22q13). We have characterized these regions for gene content and have chosen several to move further for gene characterization as described below.

Gene Candidate Characterization

C. Hutter, S. Chen [in collaboration with S. Powers, Tularik, Inc.; M. Wigler, R. McCombie, and L. Van Aelst, Cold Spring Harbor Laboratory]

Regions identified from the above studies are moving forward to the identification and the functional characterization of gene candidates. If we are to characterize the candidate genes' abilities to affect cellular growth, it is imperative that we have the full-length genes and are aware of splice variants. If one gene were being studied, this could easily be handled by more standard techniques. However, this approach will not be sufficient for multiple regions, each with multiple candidate genes. We are collaborating with Dr. Richard McCombie who has extensive experience in genomic sequencing, and his group has developed a systematic approach in which gene discovery is performed by integrating existing gene annotation and gene predictions into an experimental pipeline geared to (1) confirm the existence of genes, (2) identify the transcription start site and termination site, and (3) determine if the gene annotation is correct regarding possible splice variants.

We are also studying the gene expression of the candidates since if a gene is involved in cancer, whether it is a tumor suppressor or oncogene, it is likely that the expression of that gene is altered in a tumor. We have compiled a large set of tumors that we have analyzed and are aware of the copy number in each tumor. We then test this set of tumors to identify by quantitative reverse transcriptase (RT)-PCR to determine the expression level of the gene candidate. We are collaborating with Scott Powers' and his group at Tularik Genomics Division, to determine the expression level of several candidates. The first, *EED*, is amplified genomically in approximately 20% ovarian cancer cases. It has a polycomb domain, and a close family member (*EZH2*) is amplified in breast cancer and has been shown to have oncogenic potential. In addition, overexpression of EED in fibroblasts increases proliferation, suggesting it may also have oncogenic potential. A second gene is *PAK4*, which is amplified in pancreatic cancer in approximately 25% of cases. This gene is in the P21-activated kinase family. *PAK1*, a family member, has oncogenic potential. We will be collaborating with Dr. Linda Van Aelst on the characterization of this gene. Finally, the gene *DKK3* is deleted in pancreatic cancer, and we have also shown that its expression is decreased in pancreatic-tumor-derived samples. *DKK3* is a family member of *DKK1* and *DKK2*, all of which are antagonists of the WNT signaling pathway, members of which have been shown to have oncogenic potential and are amplified and/or overexpressed in several cancers. We intend to perform assays to determine the oncogenic potential of these candidates.

Methylation-specific Oligonucleotide Microarray Analysis

O. Dovirak, A. Brady

In addition to genetic mutation such as amplification and deletion, there are epigenetic mechanisms within the cell that are used to influence the transcriptional

activity of a gene. One such way is methylation of the cytosines present in the DNA of the transcriptional control region, which often suppresses the expression of the gene. It has been known that methylation of DNA has been involved in the silencing of gene expression in imprinting and in cancer. Recent advances including technical and the sequencing of the genome have made detection of methylation at loci more reliable and accurate. However, few methods can identify methylation changes over the entire genome. We have adapted ROMA to methylation detection oligonucleotide microarray analysis (MOMA). Currently, we are utilizing this to survey the changes that occur in cancer, but in principle, this method would have applications for identifying methylation differences involved in imprinting or other syndromes which do not involve genetic mutation.

We have taken advantage of an enzyme McrBC that cleaves at methylated cytosines, more specifically, $(G/A)^{m}C$ $(N_{40\text{-}3000})$ $(G/A)^{m}C$. Representations from tumor and normal are prepared as standard for ROMA, but the representations are treated with McrBC to remove fragments that are methylated. The samples that have are treated with McrBC are then compared to identify the fragments or regions of the genome that are differentially methylated. These regions are then searched for candidate genes.

We have tested this method on several samples and are verifying the regions identified as being differentially methylated. We are continuing to develop this method. Presently, we are redesigning the array to represent known CpG islands, as well as CG-rich regions of the genome, to make an array that is more specific for methylation detection. It is becoming clear that methylation silencing has an important role in cancer, and we intend to marry both ROMA and MOMA techniques. Both mutation and methylation have an effect on the level of gene transcription, and by analyzing those regions of the genome that have been physically mutated whether amplified or deleted and those regions of the genome that are differentially methylated, we will get a more complete picture of the cancer cell. Ultimately, all of these data will be incorporated together and analyzed alongside the clinical information to determine if any genetic or epigenetic factors correlate to clinical outcome.

Maize Mutagenesis Library Screening

R. Lucito [in collaboration with R. Martienssen and P. Rabinowicz, Cold Spring Harbor Laboratory]

The development of a high-throughput microarray screen of a maize insertion mutagenesis library is the focus of this work. The probes on the array are picked from a gene-enriched methyl-filtering library. The basis of this library relies on the fact that gene-rich regions of the maize genome are not methylated, but repetitive regions are methylated. The methyl-filtered clone library was further screened to remove clones with remaining repetitive content and redundant clones, and approximately 20,000 clones have been amplified and are being arrayed. A mutant plant library was produced by random insertion of a transposable element. DNA from these plants was isolated, and pooling was performed. Representations are prepared from these samples by PCR amplification using the sequence of the transposable element. The representations are then used for hybridization to the arrays to identify which individual plants have a gene insertion. This information will be cross-referenced to phenotypic data to identify the gene regions with transposon insertions. We are optimizing the representational procedure and labeling procedures to improve the accuracy of the technique to identify pools with mutants.

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MAMMALIAN FUNCTIONAL GENOMICS

A. Mills A. Bagchi M. Golub
S. Cheloufi W. Keyes
E. Garcia Y. Wu

Our laboratory is focused on investigating the genetic basis of human disease. We study both normal development and the tumorigenic process. By gaining insight into how specific genes normally function during embryogenesis, we are able to better understand how alterations in these genes affect pathways that cause human disease. The link between mammalian development and cancer is key in being able to design better anti-cancer therapies in the future.

We use the mouse as a model system to probe the function of human genes. The mouse provides an excellent opportunity for exploring gene function because its reproductive system, its anatomy and physiology, and even its genome are very similar to that of humans. In addition, we are able to tailor-make mouse strains in an effort to model specific human diseases. For example, we can add or remove genes, we can "cut, copy, and paste" specific regions of a chromosome, and we can even make subtle changes within a chosen gene. This ability to manipulate the mouse genome makes it possible to address a diverse array of biological questions to investigate gene function. In addition, these mouse models are invaluable for understanding pathogenesis and for designing novel treatments for human disease.

In our laboratory, we use gene targeting in embryonic stem cells to generate mouse strains that have precise modifications of the genome. We are using two different approaches to achieve this goal: (1) analyzing the role of groups of genes within specific regions of the genome, and (2) analyzing the role of *individual genes* known to be associated with human disease. The first approach uses chromosome engineering to generate mouse strains that have precise chromosome rearrangements that correlate with those found in human diseases. These mouse strains are useful for providing disease models and for determining the function of genes within specific regions of the genome. The second research emphasis in the laboratory is to perform an in-depth exploration of a single gene known to be causal in several human disease syndromes. This gene, *p63*, is related to the *p53* tumor suppressor gene—a gene that is mutated in approximately one half of all human cancers. Understanding the molecular and genetic mechanisms of *p63* action will allow us to gain insight into the processes of development, differentiation, stem cell maintenance, and cancer.

Generation of Megabase Chromosome Rearrangements: Human Chromosome *1p*

A. Bagchi, M. Golub, Y. Wu

How do large-scale chromosome abnormalities lead to disease? What are the roles of genes mapping to the short arm of human chromosome 1?

Chromosome rearrangements such as deletions, duplications, and inversions are associated with a multitude of human diseases. These chromosome abnormalities are complex because they affect large numbers of genes within specific chromosomal regions. To understand how these rearrangements cause disease, we are generating mouse strains that have the same rearrangement as those found in human patients. Defined chromosome rearrangements are made using chromosome engineering—an approach that combines the power of gene targeting with Cre/*loxP* technology. The similarities between the human and mouse genomes make it possible to generate mouse models of hereditary and spontaneous diseases. We are currently using this approach to generate models of hereditary cancer syndromes. We are focusing our efforts on human chromosome *1p*—a region containing several as yet unidentified tumor suppressor genes. A diverse array of human tumors have deletions at *1p*, suggesting that genes underlying the tumorigenic process are located in this region of the genome. We are creating models of hereditary cancer by creating mouse strains that have the same deletions as those found in human tumors. We first make specific deletions in embryonic stem cells, and then use these cells to generate mouse models that transmit the modified allele to their progeny. To make this approach feasible on a genome-wide scale, we have created a system that greatly reduces the effort required for generating

gene-targeting constructs. This system is composed of two genomic libraries of essentially pre-made gene-targeting vectors that contain all of the features required for generating Cre-induced chromosome rearrangements. In addition, constructs isolated from these libraries contain genes that alter the coat color of mice that harbor them; this feature has the advantage that mice containing a specific rearrangement in their genomes are visibly distinguishable from normal mice. This greatly reduces the cost and labor required for maintaining these mouse colonies, and also allows these models to be implemented in genetic screens.

In addition to generating mouse strains with deletions, we have also generated several large inversions that span the portion of the mouse genome that correlates to human *1p*. This work is a collaborative effort with Allan Bradley's lab at the Sanger Center. These inversion strains are currently being used in a large-scale ENU mutagenesis screen in collaboration with Monica Justice at Baylor College of Medicine. This region-specific screen will be extremely valuable for generating mouse models that will elucidate the function of disease genes mapping to human *1p*.

Analyzing the Role of Specific Disease Genes: The *p63* Gene

S. Cheloufi, E. Garcia, W. Keyes

What is the consequence of p63 *loss in vivo? How do specific* p63 *mutations cause human disease?*

The *p53* tumor suppressor gene plays an important role in human cancer. Indeed, approximately one half of all human cancers either have lost *p53* altogether or have inactivated it by mutation. Mice designed to lack *p53* are viable and develop tumors at a very early age. Thus, *p53*-deficient models are extremely valuable for investigating the molecular and genetic events associated with tumor formation.

We discovered *p63*—a gene that has striking similarity to the *p53* tumor suppressor gene. We used gene targeting to create mice that lack *p63*; these mice have severe developmental defects that affect craniofacial, limb, and skin development. Mice that inherit a nonfunctional *p63* gene are lacking all structures that are normally derived from the ectodermal cell lineage, such as hair, teeth, mammary and sebaceous glands and nails. These observations provided an important clue that led to the discovery that mutations in *p63* cause five different human developmental disease syndromes that are characterized clinically by a spectrum of malformations affecting development of the limbs, skin, and craniofacial region. How mutations in *p63* bring about the striking abnormalities in these patients is currently unknown.

We are investigating the role of *p63* in morphogenesis of the ectoderm and its related structures. Mice lacking *p63* altogether have been useful for determining that *p63* is essential for embryogenesis, but these mice have such profound developmental abnormalities that they die shortly after birth. To be able to determine how *p63* functions in the adult, specifically with regard to aging and cancer, we have generated a conditional *p63* model that allows us to first generate viable mice, and then to ablate *p63* within specific tissues at particular stages of development. Using this approach, we are able to inactivate *p63* within specific tissues. This is the first system that provides a tool for directly analyzing the role of *p63* in the adult.

We have also generated mouse models that mimic the human developmental disease syndrome Ectrodactyly, Ectodermal dysplasia, Clefting (EEC) syndrome. EEC is caused by specific mutations in the DNA-binding domain of *p63*; interestingly, these mutations correlate precisely with cancer-causing mutations in the *p53* gene. To model EEC, we used gene targeting to replace the normal copy of *p63* with a version of *p63* that has an EEC mutation. Mice carrying the EEC mutation have a phenotype that correlates with the clinical features found in EEC patients (Fig. 1). Further

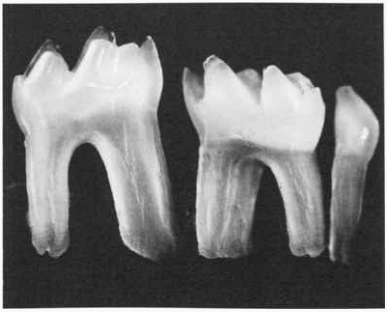

FIGURE 1 EEC mice display severe tooth defects. EEC patients with *p63* mutations often have an abnormal number of teeth. Similarly, mice engineered to have the same mutation in *p63* that causes human EEC have hyperdontia (*extra teeth*).

characterization of this model will allow us to more thoroughly understand the underlying cause of the pathogenesis of EEC, and will form a basis for better treatment of patients afflicted with this disease.

Within the last year, we have also collaborated with Dennis Roop's lab at Baylor College of Medicine in generating mouse models that overexpress specific isoforms of *p63*. By creating "gene switch" transgenic mice that use the keratin 14 promoter to overexpress *p63* specifically within proliferating keratinocytes of stratified epithelia of the skin, we found that *p63* has a dual role in vivo: It initiates epithelial stratification during embryogenesis and maintains the proliferative potential of basal keratinocytes in the mature epidermis.

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In Press

Koster M.I., Kim S., Mills A.A., DeMayo F.J., and Roop D.R. 2004. *p63* is the molecular switch for initiation of an epithelial stratification program. *Genes Dev.* **18:** 126–131.

A brief break from the bench. The Mills lab attends the Inaugural Symposium for the Developmental Biology Program at the Sloan-Kettering Institute at Memorial Sloan-Kettering Cancer Center in New York. Elvin Garcia (*second from left*), a graduate student in the lab, presented his unpublished work to colleagues. Other members of the lab that attended this meeting include Bill Keyes (*left*), Alea Mills (*middle*), Anindya Bagchi (*second from right*), and Ying Wu (*right*).

TUMOR NEOVASCULARIZATION

V. Mittal J. Egan
S. Gupta
R. Kumar

The growth and metastasis of a majority of tumors depends on the formation of new blood vessels by a process called angiogenesis. The process by which endothelial cells (ECs) neovascularize a tumor to support its continuous growth and metastasis is an area of intense investigation. Identification of the molecular pathways that direct EC proliferation, migration, and functional vessel formation in tumors is of fundamental importance for developing rational antiangiogenic therapies.

Genetic approaches have established an absolute requirement for helix-loop-helix (HLH) *Id1* and *Id3* transcription factors in the formation of functional tumor neovascularization. Given this critical function, it is surprising that to date no systematic search for *Id*-regulated genes has been performed. Our research is geared toward identifying downstream components of the *Id* gene network, analyzing the mechanism of action of these components, and determining their relative impact on EC proliferation, migration, and functional neovascularization in both xenografts and spontaneously arising tumors in vitro and ultimately in vivo.

We apply functional genomic approaches, such as genome-wide transcription profiling and RNA interference (RNAi)-mediated gene silencing, to mouse genetic models to dissect *Id*-mediated cellular pathways. Our major focus is to determine at the molecular and genetic levels the mechanism by which ECs neovascularize a tumor, to support its continuous growth and metastasis. The identification and functional analysis of *Id* transcriptional targets will not only provide fundamental insights into the role of *Id* as a transcription modulator, but also impact development of rational antiangiogenic therapeutic interventions that disrupt *Id*-mediated tumor growth in humans.

Role of *Id* in Tumor-mediated Angiogenesis

S. Gupta [in collaboration with M. Ruzinova, Memorial Sloan-Kettering Cancer Center]

Angiogenesis results from the outgrowth of neighboring preexisting vessels and the recruitment of bone-marrow-derived endothelial precursor cells (EPCs). To understand these processes, we use a mouse model that lacks transcription factors *Id1* and *Id3* and exhibits defective tumor vasculature leading to impaired growth and metastasis.

The HLH Id proteins are key players in regulating angiogenesis in a majority of tumors. In adult mice, *Id1* and *Id3* are overexpressed in the endothelium of the tumor vasculature but not in the surrounding tumor cells or in preexisting vessels. *Id1* and *Id3* are also essential for the expansion and mobilization of bone-marrow-derived vascular endothelial growth factor receptor 2 (VEGFR2) and VEGFR1-positive cells from the bone marrow to the sites of tumor neovascularization. Loss of *Id1* and *Id3* causes severe defects in tumor-induced neovascularization and impairs growth and metastasis of both xenografts and spontaneous tumors. What are the downstream consequences of *Id* loss that lead to the observed defects in postnatal angiogenesis? Since *Id* itself controls the transcription of other genes, it will be important to determine both the immediate-early transcriptional effect of *Id* loss and secondary transcriptional effects to fully understand the phenotype. Additionally, *Id*s and their target genes would be ideal candidates for antiangiogenic therapy. Our broad specific aims are to identify *Id*-regulated genes in the ECs of tumors, analyze their mechanism of action, and determine their relative impact on tumor progression.

In last year's Annual Report, we mentioned the identification of the components of an integrin pathway that is up-regulated in the endothelium, infiltrating the *Id* wild-type spontaneously growing tumors. Candidate genes, including integrin α6, integrin β4, fibroblast growth factor receptor-1 (*FGFR1*), metalloprotease-2 (*MMP2*), and laminin 5, are all up-regulated in the presence of *Id*. Interestingly, it is known that *FGFR2* engagement of *FGFR1* leads to an up-regulation of α6β4, and *MMP2* cleaves laminin 5 to produce a ligand for α6β4. Although α6β4 integrin expression in ECs has been documented, not much is known about their function in vivo. However, α6β4 integrin promotes stable adhesion of epithelial cells to the extracellular matrix (ECM) during proliferation and

migration of invasive epidermal carcinoma, suggesting that it may also facilitate EC invasion and migration across the ECM during tumor vascularization (note that *MMP2*, another key player for ECM degradation is also up-regulated in *Id* wild-type endothelia). Identification of integrins as *Id* targets is not unexpected, given the central role of integrins αVβ3, αVβ5, and α5β1 in supporting pathological angiogenesis. Blocking the activity of these integrins results in a dramatic inhibition of angiogenesis. In our screens, only α6 and β4 integrins were differentially regulated, increasing the probability that they may be regulated by *Id*. We hypothesize that loss of *Id* affects several players in this pathway and thus prevents functional vessel formation by the EC. This hypothesis is currently being experimentally tested both in in vitro and in vivo.

We performed transcription-profiling analysis directly on intact tumors extracted from *Id* wild-type and *Id* mutant animals. It is possible that the nonendothelial components of tumors decrease signal/noise ratios for endothelium-specific genes, and therefore, subtle changes in gene expression can be missed. We thus purified ECs from lymphoid lesions of *Id* wild-type and *Id* mutant animals and analyzed *Id* gene expression profiles from a relatively pure population of cells (the purity of isolated ECs was 90–95% as assessed by immunofluorescence with CD31 and von Willebrand factor). A number of other angiogenesis-related genes were down-regulated twofold to sixfold in the $Id1^{-/-}$ ECs such as members of ephrin, the insulin-like growth factor (IGF), and transforming growth factor β (TGF-β) families, as well as genes encoding ECM proteins and cell adhesion factors. In addition, sixfold up-regulation of Hif1α was detected in $Id1^{-/-}$ ECs. Ephrin A1 and its receptor EphA2, which are both expressed in xenograft and spontaneous tumor vasculature, have been shown to induce EC migration and in vitro capillary assembly, as well as corneal and tumor neovascularization. *IGF2* and its receptor, down-regulated in $Id1^{-/-}$ ECs, also possess angiogenic properties. *IGF2* induces EC migration and tube formation as well as up-regulates expression of *MMP2*. Thus, down-regulation of these factors in the $Id1^{-/-}$ ECs may be contributing to the observed phenotype. Up-regulation of Hif1α mRNA in the *Id* mutant endothelium suggests a possible direct transcriptional effect since the Hif1α promoter has four conserved E-box consensus sequences, which are recognized by basic HLH transcription factors.

Current experimental work is geared toward establishing connections between different angiogenic pathways disregulated in an *Id* mutant background in order to gain a more thorough understanding of their contribution to the final phenotype. Inhibitory short hairpin RNAs (shRNAs) to *Id* target genes will be introduced into ECs, and the impact of loss of gene function on blood vessel formation will be monitored in rapid in vitro matrigel assays and in more physiologically relevant in vivo model systems. We are confident that this strategy will work because combined suppression of *Id1* and *Id3* results in a dramatic phenotypic change, associated with defects in EC migration and tube formation (see below). Alternatively, we will attempt rescue of the *Id* loss-of-function phenotype by introducing and activating potential *Id* downstream targets in *Id1*, *Id3* null ECs. Together, these approaches will determine the role of *Id* downstream components in neovascularization.

We are also examining the role of *Id1* and *Id3* in the mobilization and recruitment of the bone marrow precursor to the sites of the tumor and subsequent formation of the neovasculature. Delivery of shRNAs to the bone marrow using lentiviruses will be employed to inhibit the activity of genes, which may contribute to the *Id* phenotype. Our approach provides a flexible platform where effective shRNA probes can be rapidly generated against any gene (see below) and delivered in vivo directly to the site of the tumor vasculature. Site-specific expression of shRNA will be achieved by employing an innovative design where an EC-specific gene regulatory element drives expression of shRNA which mediates suppression of genes that are deregulated specifically in the tumor endothelium. This strategy ensures minimal affects of gene suppression on normal vasculature or surrounding tumor cells. Overall, our approach has unlimited potential in inactivating any proangiogenic gene in vivo.

Identification of Effective siRNAs for Gene Silencing and Their Inducible Delivery in Mammalian Cells

R. Kumar, S. Gupta

Although improved rational designs have enhanced the ability to generate effective small interfering RNAs (siRNAs), they do not still ensure that a single predicted siRNA will silence the target gene with utmost accuracy. To utilize RNAi for systematically suppressing expression of any *Id* target gene, we have developed a screening method for the identification of

the most potent siRNA. The efficacy of siRNA sequences is monitored by their ability to reduce the expression of cognate sequences in an ectopically expressed target mRNA that is fused to a reporter gene in conventional transfection experiments. The approach allowed not only the identification of the most effective siRNAs, but also those that display partial suppression of target gene expression. Such siRNAs would be useful where varying degrees of gene silencing might result in unique phenotypes. To automate this screening process so that effective siRNA probes can be identified in large-scale screens, a microarray-based cell transfection ("RNAi microarrays") method was developed. Using RNAi microarrays, we demonstrated an unlimited potential of this approach in high-throughput screens for identifying effective siRNA probes for silencing genes in mammalian systems. The screening method was used to identify effective shRNA probes for murine *Id1* and *Id3*, and sRNA-mediated loss of *Id* gene function in adult ECs phenocopied the angiogenic defect observed in *Id* knockout mice in matrigel assays. ECs stably expressing *Id1*, *Id3,* and combined *Id1* and *Id3* shRNAs marked with a green fluorescent protein (GFP) reporter appeared to lose their flat endothelium-like morphology and ability to form endothelial tubes in the VEGF impregnated three-dimensional matrigel. Thus, shRNA-mediated loss of *Id* gene function in adult EC phenocopies the angiogenic defect observed in the *Id* knockout mice.

The existing methods of gene suppression by the constitutive expression of shRNAs allow analysis of the consequences of stably silencing genes, but significantly limits the analysis of genes essential for cell survival, cell cycle regulation, and development. Besides, gross suppression of a gene over longer periods can result in compensatory or even nonphysiological responses that mask the true biological consequence of a functional knockdown. Generating inducible regulation of RNAi should be able to circumvent this problem. Several groups have developed inducible siRNAs whose expression is controlled by the tetracycline- or doxycycline-regulated form of RNA polymerase III (pol III) U6 or H1 promoter. A limitation of the tetracycline-inducible system is a relatively high background of expression in the uninduced state in certain cell lines, and toxicity problems in some cases limit studies involving both cultured cells and animals. Considering these limitations, we have recently developed ecdysone-inducible synthesis of shRNAs under the control of a modified pol III-specific U6 promoter in both human and murine cells.

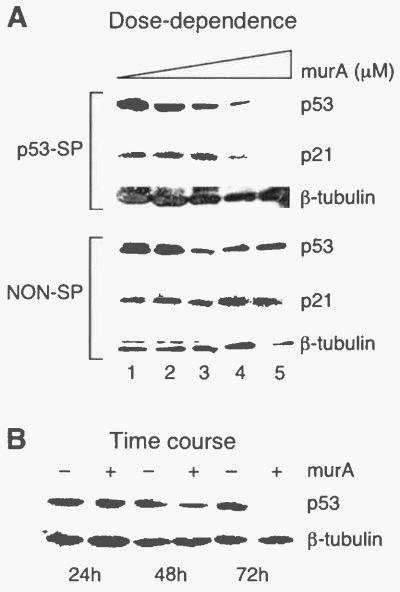

FIGURE 1 Response of ecdysone-inducible RNAi. (*A*) Stable cell lines carrying p53-specific shRNA (p53-SP) and nonspecific shRNA (NON-Sp) were induced with 0.0 (lane *1*), 0.5 (lane *2*), 2.0 (lane *3), 3.0 (lane* 4), and 5.0 (lane *5*) μM *murA*. Whole-cell extracts were prepared after 72 hr and analyzed by western blotting for p53, p21, and β-tubulin (control). Fold-reduction in p53 protein level is 30% (lane *2*), 64% (lane *3), 90% (lane* 4), and >95% (lane *5*) relative to uninduced sample (lane *1). (B*) Time course of ecdysone-inducible RNAi. Stable cells carrying shRNA for p53 were induced with 5 μM *murA* and analyzed for p53 levels at indicated time points, and fold-reduction of p53 protein level is 60% (+*murA* at 48 hr), whereas (+*murA* at 96 hr) is >95%. Fold-reduction of protein level was based on densitometric measurement.

Cells containing stably integrated shRNA expression constructs demonstrate stringent dosage- and time-dependent kinetics of induction with undetectable background expression in the absence of the inducer ecdysone (Fig. 1). Inducible suppression of human p53 in glioblastoma cells shows striking morphological changes and defects in cell cycle arrest caused by DNA damage, as expected. A major limitation of constitutive shRNA expression systems is the irreversible suppression of gene expression that could result in nonphysiological responses. Remarkably, the inducibility is reversible following withdrawal of the inducer as observed by reappearance of the protein and a restoration of the original cell phenotype (Fig. 2). The generation of an inducible pol III promoter opens up the possibility of tissue- or cell-specific regulation of shRNAs by virtue of expressing the GAL4 *trans*-activator under the control of defined tissue-specific promoters. This is likely to have widespread applications in both cultured cells and living animals.

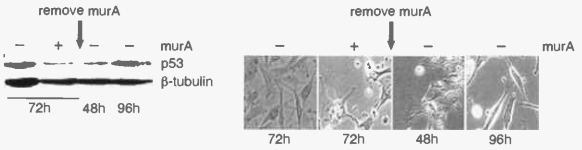

FIGURE 2 RNAi-mediated inducible gene suppression is reversible. (*Left*) Western blot analysis shows 93% fold-reduction in p53 protein levels in cells induced with 5 μM *murA* (+*murA*) relative to uninduced (–*murA*) at 72 hr. Following *murA* removal, the fold-recovery in p54 levels is 20% (48 hr) and >90% (96 hr). (*Right*) Phase contrast microscopy shows morphology of U87MG cells either uninduced (–*murA*) or induced with 5 μM *murA* (+*murA*) for 72 hr and at 48 and 96 hr following *murA* removal.

Role of an Immunomodulatory Drug in Maturing Human Dendritic Cells

J. Egan [in collaboration with Immuno-Rx, Farmngdale, New York]

Dendritic cells (DCs) are antigen-presenting cells that have vital dual roles in initiating primary (innate) immune responses as well as being critical mediators and directors of subsequent adaptive immune responses. Following exposure to a pathogen, DCs rapidly ingest and display antigens from the foreign organism and undergo maturation into potent antigen-presenting cells, capable of efficiently stimulating T cells. Maturation is coupled to their migration to lymphoid organs where they encounter naïve T cells and initiate an immune response, including cytokine and chemokine production. Their phenotypic and functional characteristics are intimately linked to their stage of maturation. We are currently investigating the immunomodulatory compound inosine-5′-methyl phosphate (MIMP) and its role in dendritic cell maturation. We are using a DC system derived from human peripheral blood monocytes that are induced to differentiate into immature DCs following incubation with interleukin-4 and granulocyte-macrophage colony-stimulating factor. Flow cytometry analysis of surface antigen markers of those cells treated with MIMP typically revealed an increase in the kinetics of the transition from monocytes to immature dendritic cells, as well as an increase in the maturation-specific marker CD86. MIMP also induced morphological changes in those cells that correlate with a mature phenotype. Additionally, functional studies revealed that MIMP-treated DCs more effectively activate lymphocytes than untreated cells. DNA microarray analysis is being performed to attempt to elucidate the global gene expression profile changes induced by MIMP. A further understanding of the molecular pathways involved in DC maturation as well as compounds that regulate this process will allow for the design of more powerful vaccines to combat infectious diseases and cancer.

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MAMMALIAN CELL GENETICS

M. Wigler J. Alexander, J. Allen, K. Chang, M. Chi, J. Douglas, D. Esposito, H. Grasmo-Wendler, I. Hall, J. Hicks, J. Healy, L. Muthuswamy, U. Nath, N. Navin, A. Reiner, M. Riggs, L. Rodgers, J. Sebat, E. Thomas, J. Troge, J. West

I liked the start of my section of last year's annual report so much, that I have decided to use the first two paragraphs again. I apply the adage I first heard from Sol Spiegelman, "any thing worth saying, is worth saying twice." Here it is.

It is a poor and unnecessary gamble to act as though either our theory or our knowledge of cancer is complete. Future progress in detection, prognosis, and treatment of cancer will depend on the accuracy and completeness of our understanding of its specific molecular causes. This knowledge is likely to become increasingly important as cancers, or suspected cancers, are detected at earlier and earlier stages.

There are simple tests for the completeness of our understanding of how cancers survive in and kill their hosts. If our knowledge were complete, we would see a plateau in the number of genes commonly found mutated in cancers. If the principles were few, even advanced cancers with a large number of accumulated genetic lesions would show only a small number of commonly affected pathways. It follows from this that if mutation in a single gene were sufficient to affect a given pathway, then even advanced cancers would show only a small number of commonly affected genes, the remainder of lesions being more-or-less random.

To approach the question of a "complete" understanding of cancer, we have developed a microarray-based method, called ROMA (representational oligonucleotide analysis). ROMA is based on part on our previous technique called RDA (representational difference analysis). Unlike cDNA microarrays, which can describe the "transcriptional" state of the cell, ROMA measures changes in "gene copy number" at loci that undergo amplifications and deletions, hallmarks of oncogenes and tumor suppressor genes, respectively. Although there are many other possible mechanisms that alter critical genes, such as point mutations, many if not most oncogenes and tumor suppressor genes will eventually be found in the types of lesions that we can readily detect. In principle, our method can also detect changes in the methylation of DNA, imbalanced translocations, origins of replication, and long-range features of chromatin structure.

Our basic assumption is that if a locus is recurrently found altered in cancers, that region harbors a candidate cancer gene. Therefore, the application of our method to a large series of cancers, and the comprehensive comparative analysis of such data, should reveal the position and number of candidate cancer genes in cancers. We have progressed reasonably on the task of collecting the data from cancer cells that will lead to the definition of these recurrently abnormal regions.

Using our methodology, we have also determined that there are many copy-number differences in the human gene pool, i.e., large regions of the human genome that are present in individuals in unequal amounts. These variations are germ-line, Mendelian in inheritance, distributed throughout the genome, and rich in genes. Many are common polymorphisms, found in almost equal numbers throughout the human gene pool. It seems likely to us that many of these regions are under selective pressure and will be shown to be associated with disease resistance and sensitivity. In any event, it is necessary to make a database of these variations, so as not to mistake them for cancer lesions, and we are well under way to accomplish this.

THE TECHNOLOGY

The basis of our ROMA technology has been explained over the past years. It involves making complexity-reducing representations of genomic DNA and hybridizing these representations to microarrays of oligonucleotide probes designed informatically, from the published human genome assembly, to be complementary to the representations (Lucito et al. 2003). The probes are chosen from the genome so that they have a minimal overlap with unrelated regions of the genomes. The method for making this computation was published this year (Healy et al. 2003) and has been a tool in the discovery of a new feature of mam-

malian genomes by a graduate student, Elizabeth Thomas (see below). The algorithms allow counts of exact matches of sequences of any length throughout a sequenced genome and are based on a Burrows-Wheeler transform of the genome sequence.

We use two forms of oligonucleotide microarrays: the printed form that we make ourselves, and a form in which oligonucleotides are synthesized in situ on the array surface using laser-directed photochemistry. A company called NimbleGen Systems makes the latter, and their technology has given us substantially greater flexibility in the design of arrays and the selection of representations. Pictures of these microarrays are shown in Figure 1, with the printed array on the left and the Nimblegen array on the right. We have shown that each format yields very similar measures, probe for probe. This work was conducted in collaboration with Robert Lucito here at CSHL, and a report of our findings was published this year (Lucito et al. 2003). With NimbleGen, we typically array 85,000 probes (85K format), averaging one probe per 30kb, but even greater densities can now be achieved, nearly 200,000 probes per chip. To facilitate our close relationship with NimbleGen, which fabricate their chips in Reykjavik, CSHL has set up an Icelandic subsidiary with personnel trained by our laboratory.

CANCER LESIONS

We have applied our method to both tumor biopsies and cancer cell lines and have observed gross chromosomal copy-number alterations, and highly localized amplification, imbalanced chromosome breaks, and deletions. In the latter case, we expect that we have observed both hemizygous and homozygous deletions. In our data analysis, we have used algorithms for statistical segmentation. The first version of this was designed by Adam B. Olshen and E.S. Venkatraman of the Memorial Sloan-Kettering Cancer Center (Olshen et al. 2003). Subsequent versions were designed in collaboration with Kenny Ye of the Department of Applied Mathematics and Statistics at Stony Brook University, and are based on minimization of variance and an assumption of log normal distribution of ratio data.

We observe a large number of lesions, of varying sizes, per cancer. Breast cancers appear to divide into two types: those with large numbers of genomic changes and those with very few. The former also further divide into two types: those that appear to be evolving lesions slowly and those that appear to be evolving rapidly. Virtually all of the known lesions have been seen, as well as many new ones. We are now in the stage of accumulating data and performing comparative analysis. We expect this study to give us a good estimate of the number of pathways involved in the development of breast cancer, while identifying those major pathways.

NORMAL VARIATION

We have applied our method to the comparison of normal genomes and discovered that there are a large number of extensive regions of copy-number variation

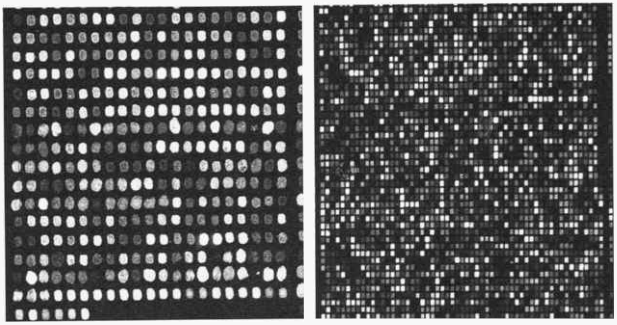

FIGURE 1 Two forms of oligonucleotide microarrays.

between any two humans. We have made 24 comparisons and have noted on the order of 240 differences at about 80 specific loci. Of these, nearly half of differences are recurrent, indicating that they arise from common polymorphisms in the human gene pool. The regions showing copy number are rich in genes and are distributed fairly uniformly across the human genome, with the exception of the X chromosome. We have confirmed these variations by polymerase chain reaction (PCR), interphase fluorescence in situ hybridizaton (FISH) (a collaboration with Anders Zetterberg of the Karolinska Institute, Sweden, and Barbara Trask, University of Seattle, Washington), and independent ROMA using *Hin*dIII (as opposed to *Bgl*II) representations. The regions arise by both polymorphic gene duplications and deletions. These regions must be categorized, as otherwise they will be mistaken for recurring cancer lesions in our cancer surveys. Furthermore, these normal variations may be associated with inherited disease susceptibility or resistance. In a collaboration with Conrad Gilliam of Columbia University College of Physicians & Surgeons, we are conducting a large survey of normal genomes and genomes from families with children with autism syndromes.

CANCER GENES

In the previous year, we have described the discovery of new oncogenes (Mu et al. 2003) and tumor suppressors (Hamaguchi et al., *Proc. Natl. Acad. Sci. 99:* 13467 [2002]) using RDA or array-based methods. Although this work is continuing by our collaborators, and we are continuing research on PTEN, we are focusing presently on the accumulation of massive amounts of copy-number data from cancer cell lines and tumors, and we have demonstrated our ability to obtain this information from clinical material archived as either frozen or formalin-fixed. We have seen promising cancer genes in tumor amplifications, encoding proteins such as kinases, transcription factors, receptors, and antiapoptotic factors and have observed interesting candidate tumor suppressor genes in regions of loss, encoding cellular components such as checkpoint control proteins and ubiquitin ligase subunits. But our general approach has been to hold off on the difficult task of functional validation until we have collected a sufficient amount of copy-number profiles to winnow the candidates and determine priorities. In future work, it will also be a priorty to correlate patterns of gene loss and gain with clinical outcomes.

GENOMES AND EVOLUTION

In collaboration with Bud Mishra and Will Casey at the Courant Institute for Applied Mathematics at New York University, we developed the algorithmic basis for the use of microarray hybridizations to map genomes (Casey et al., *Lect. Notes Comput. Sci. 2149:* 52 [2001]). Joe West and John Healy in my lab collected a full set of data in a model organism, *Schizosaccharomyces pombe*, which has a complete sequence assembly and is putting these ideas to test. We are finding that the empirical data fit the mathematical model closely, and apart from the difficulty of assembling centromeric and telomeric regions, and additional hybridization data needed because of "noise" in the system, the probes by and large map into long linear or only slightly branched structures. We predict from this work that array hybridization is a feasible way to validate a sequence assembly or to obtain a rough "local" probe map of a new organism.

Elizabeth Thomas, a Watson school graduate student, in collaboration with John Healy here at CSHL, Nathan Srebro at the Massachusetts Institute of Technology, and Bud Mishra of the Courant, has used our exact matching algorithms to discover a new and fundamental feature of genomes. Mammalian genomes are densely populated with "doublets," short duplications between 25 and 100 bp, distinct from previously described repeats. Each doublet is a pair of exact matches, separated by some distance. The distribution of these intermatch distances is strikingly nonrandom. One interesting characteristic of nearby doublets is that both exact matches tend to occur in the same orientation. By comparing doublets shared in human and chimp or mouse and rat, we can see that at least nearby doublets seem to arise by an insertion event that does not affect the neighboring sequence. Most doublets in humans are shared with the chimpanzee, but many new pairs, especially adjacent ones, arose after the divergence of the species. New doublets are most likely to be adjacent, whereas older doublets are almost equally likely to be nearby or adjacent, indicating that adjacent doublets may be unstable, disappearing over time. A genomic mechanism that generates short, local duplications while conserving polarity could have a profound impact on the evolution of regulatory and protein-coding sequences.

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Ira Hall

In their efforts to understand and treat cancer, CSHL scientists are exploring how cells become cancerous and searching for specific vulnerabilities in cancer cells that might provide targets for effective therapeutic strategies. To comprehend the complex mechanisms by which cells form tumors, researchers study the normal properties of healthy cells and determine how these properties are altered in cancer cells.

To better grasp how genes direct cell function, David Spector's lab has engineered a cell line that allows them to watch DNA, RNA, and proteins in concerted action. Carefully designed molecules allow them to see RNA transcribed from DNA and then to visualize proteins translated from the RNA. In this way, Spector and his colleagues have provided the first glimpses of the entire process of gene expression in living cells.

Arne Stenlund focuses on the events that trigger DNA replication and is working to unravel the crucial first steps during the replication of papillomavirus DNA. Papillomavirus is associated with virtually all cases of cervical cancer. By studying the biochemical and structural changes that initiate viral DNA replication, Stenlund has revealed key details not only about this cancer-causing virus, but also about the process of DNA replication in general. Moreover, these studies have uncovered specific molecular targets for the development of drugs to prevent or treat cervical cancer.

Bruce Stillman's lab explores events that initiate cellular rather than viral DNA replication. One focus of their research is a protein complex called ORC that attaches to DNA and both triggers DNA replication and silences gene expression. Recently, Stillman's lab has discovered new roles for ORC in chromosome segregation (the sorting of chromosomes during cell division such that each cell receives a complete set of chromosomes) and in cytokinesis (the process in which cells divide as one cell becomes two). As the researchers piece together the multiple roles of ORC and its various protein partners, they are uncovering vital clues concerning how the control of DNA replication and cell proliferation is lost in cancer cells. Stillman's group also studies how the large-scale architecture of chromosomes (called chromatin) is duplicated, and how chromatin assembly is coupled to DNA replication. They have discovered that when a protein required for chromatin assembly is depleted from human cells, DNA replication ceases, a system that monitors DNA damage is triggered, and the cells die due to a protective mechanism called programmed cell death. That finding is intriguing because it reveals a connection between chromatin assembly and the replication of human DNA, and it suggests that the proper assembly of newly replicated DNA into chromatin is necessary for DNA replication to continue.

If a telephone cord is twisted continuously in either direction, after a few turns, kinks or "supercoils" will form. Similar kinds of supercoils form in DNA within cells as it participates in many processes, but the presence of supercoils is strictly regulated by enzymes and other proteins that can add or remove them. CSHL Fellow Terence Strick uses single-molecule biophysics to directly measure supercoiling and other structural transitions in DNA and, in turn, to deduce the properties of enzymes and other proteins that alter DNA structure.

Most cancers arise from epithelial cells, which line organs and body cavities. Senthil Muthuswamy's lab is investigating how cells in healthy breast epithelium form hollow spherical structures, and how cancer-causing genes, called oncogenes, cause the cells in these structure to proliferate out of control and metastasize.

David Helfman's lab is working to understand how oncogenes disrupt a cell's cytoskeleton, the internal framework that gives a cell its shape and strength and controls its interactions with neighboring cells. Cancer is due in part to the loss of such controlled interactions among neighboring cells, a loss of control, which enables cells to break free from their biological moorings and become metastatic.

Linda Van Aelst's lab is trying to understand how genetic mutations cause changes in intracellular signaling molecules which then trigger disease. They have been working with proteins called Ras and Rap, known to affect cell growth and to play a normal role in wound healing, but also contribute to can-

cer when mutated. This year, in collaboration with Roberto Malinow's lab, they discovered that the same proteins also have roles in brain development, learning, and memory. The discovery helps explain why mutations in another protein that controls Ras function called NF1 can lead both to cancer and to cognitive defects. Van Aelst has also revealed the involvement of proteins called RhoA GTPase and oligophrenin-1 (which regulates RhoA GTPase) in X-linked mental retardation.

Tatsuya Hirano's lab studies the function of proteins that act to modulate large-scale chromosome structure and behavior. One of these proteins, called condensin, is required for the condensation or compaction of chromosomes that occurs prior to cell division. Hirano's team was working with a type of condensin that is found in organisms from yeast to humans. But this year they discovered that vertebrate cells have a second type of condensin (condensin II) that helps shape chromosomes. By studying this newly discovered protein, Hirano's group hopes to better understand how the architecture of chromosomes is established and maintained.

Nicholas Tonks' lab studies how enzymes called protein tyrosine phosphatases (PTPs) function to counteract the affects of growth factors and hormones, and thereby help control cell growth. Some PTPs have been linked to diseases including cancer and diabetes. As one of several projects this year, Tonks' lab continues to study the involvement of "dual specificity" phosphatases, including JSP-1 and DEP-1, in cancer and other diseases. In addition, they are exploring the importance of a novel form of protein modification as a general physiological mechanism for regulating PTP function.

Christian Speck, postdoc in the Stillman Lab

THE CYTOSKELETON AND ONCOGENIC TRANSFORMATION

D.M. Helfman E. Araya S.-W. Lee
L. Connel G. Pawlak
E. Kim

We are interested in understanding the molecular mechanisms underlying the regulation and function of the actin cytoskeleton in oncogenesis. Alterations in the actin-based cytoskeleton are an established part of the neoplastic phenotype. These alterations result from activation of specific signaling pathways that are part of the oncogenic program. Oncogene-mediated changes in actin filament dynamics and associated adhesive interactions contribute to enhanced motility and invasiveness of tumor cells. These changes in the cytoskeleton are a result of changes in the expression of both specific cytoskeletal proteins and signaling proteins, e.g., small GTPases and kinases. The changes in the expression of cytoskeletal proteins are more than simply a generic consequence of cell reorganization characteristic for oncogenic transformation. For example, work by our lab and others has shown that ectopic expression of specific cytoskeletal proteins can suppress many features of transformation, including disruption of microfilament bundles and focal adhesions, loss of contact-inhibited cell growth, ability to grow in soft agar, and tumorigenicity in nude mice, indicating that the loss of specific cytoskeletal proteins has a direct role in oncogenesis. However, the mechanisms by which specific components of actin structures are targeted and deregulated by oncogenes are poorly understood, and, more importantly, how the accompanying changes in actin filament organization contribute mechanistically to oncogenesis remains to be established. Below is a brief description of our studies during the year to better understand the regulation and role of the actin cytoskeleton in oncogenesis.

Alterations in Tropomyosin Isoform Expression in Human TCC of the Urinary Bladder

G. Pawlak [in collaboration with T.W. McGarvey, T.B. Nguyen, J.E. Tomaszewski, R. Puthiyaveettil, and S.B. Malkowicz, University of Pennsylvania]

Previous studies of transformed rodent fibroblasts have suggested that specific isoforms of the actin-binding protein tropomyosin (TM) could function as suppressors of transformation, but analysis of TM expression in patient tumor tissue is limited. The purpose of this study was to characterize expression of the different TM isoforms in human transitional cell carcinoma (TCC) of the urinary bladder by immunohistochemistry and western blot analysis. We found that TM1 and TM2 protein levels were dramatically reduced and showed more than 60% reduction in 61% and 55% of tumor samples, respectively. On the other hand, TM5, which was expressed at very low levels in normal bladder mucosa, exhibited aberrant expression in 91% of tumor specimens. The western blot findings were confirmed by immunohistochemical analysis in a number of tumors. We then investigated the mechanism underlying TM expression deregulation in the T24 human bladder cancer cell line. We showed that levels of TM1, TM2, and TM3 are reduced in T24 cells, but significantly up-regulated by inhibition of the mitogen-activated protein kinase-signaling pathway. In addition, inhibition of this pathway was accompanied by restoration of stress fibers. Overall, changes in TM expression levels appear to be an early event during bladder carcinogenesis. We conclude that alterations in TM isoform expression may provide further insight into malignant transformation in transitional cell carcinomas of the bladder and may be a useful target for early detection strategies.

The Actin Cytoskeleton and Apoptosis

L. Connell

Adhesion of cells to the extracellular matrix (ECM) via integrins generates signals required for cell growth and survival. When normal epithelial cells are deprived of these survival signals, i.e., when detached from the ECM, they undergo a specialized form of apoptosis, termed anoikis. In contrast, transformed cells are resistant to anoikis, presumably through constant activation of survival signals.

The cytoskeleton has an important role in the reg-

ulation of cellular functions linked to transformation, including proliferation, anchorage-independent cell growth, and apoptosis. Actin filaments have a critical role in the activation of integrins, and subsequent generation of survival signals. Myosin light-chain kinase (MLCK) and Rho-kinase (ROCK) regulate actin filament assembly and myosin II, and myosin II is known to have a role in stress fiber formation and integrin signaling. To determine if myosin and actin contribute to survival signals, we studied whether inhibition of myosin or disruption of actin filaments leads to apoptosis in normal and transformed epithelial cells (MDCK, MDCK Ras, MCF-7, MCF10A, MCF10 Ras, and DU145 cells). Using agents that target actin filaments, we found that both normal and transformed epithelial cells undergo apoptosis following disruption of actin filaments. Using agents that inhibit myosin II, we studied the role of myosin-dependent pathways on cell survival. Interestingly, treatment of cells with of myosin II or MLCK inhibitors led to apoptosis in both normal and transformed cells. In contrast, treatment of cells with the ROCK inhibitor did not induce apoptosis. These results suggest that actin filaments and myosin II have an important role in the generation of survival signals. Finally, our results suggest that MLCK has an essential role in the regulation anchorage-independent cell growth of transformed cells and further highlight the functional diversity between Rho-kinase and MLCK-dependent function.

Cytoplasmic $p21^{Cip1}$ Is Involved in Ras-induced Inhibition of the ROCK/LIMK/Cofilin Pathway

S.W. Lee

$p21^{Cip1}$ is best known for its ability to directly block the kinase activities of a broad range of cyclin/CDK complexes in response to antimitogenic signals or DNA damage. Despite its function as a cell cycle regulator, elevated levels of $p21^{Cip1}$ in the cytoplasm of tumor cells have been reported to be critical for promoting cell transformation and survival. Strikingly, the level of $p21^{Cip1}$ expression is highly increased in various human cancers such as breast cancer, bladder cancer, pancreatic cancer, and glioblastoma. It remains unclear how elevated cytoplasmic $p21^{Cip1}$ might contribute to tumorigenesis. During the past year, we have found that expression of cytoplasmic $p21^{Cip1}$ is an essential factor in the signaling pathways that mediate Ras-induced alterations of the actin cytoskeleton. We found that oncogenic Ha-RasV12 contributes to the alterations in actin filament dynamics by inducing cytoplasmic localization of $p21^{Cip1}$, which uncouples Rho-GTP from activating the ROCK/LIMK/cofilin pathway by inhibiting Rho-kinase. Our findings define a novel mechanism for coupling the cytoplasmic $p21^{Cip1}$ to the control of actin cytoskeleton remodeling by oncogenic Ras. Furthermore, our studies demonstrate that localization of $p21^{Cip1}$ to the cytoplasm in transformed cells contributes to pathways that favor not only cell proliferation, but also cell motility, thereby contributing to invasion and metastasis.

Characterization of the Metastasis-associated Protein, S100A4

E. Kim

Elevated levels of S100A4, also referred to the metastasis-associated protein, is associated with metastatic tumor progression by enhancing cell motility. S100A4 is a calcium-binding protein that is known to form homodimers and interact with several proteins in a calcium-dependent manner, including tropomyosin and myosin IIA. The interaction of myosin IIA is believed to disrupt myosin self-assembly and inhibit protein kinase C or casein kinase II phosphorylation of the heavy chain. Because of its proposed role in the regulation of myosin function, we carried out an extensive analysis of the myosin-II-binding functions of S100A4, including an analysis of its calcium-binding domains, dimerization properties, and cellular localization. Interestingly, our studies show that S100A4 localizes to lamellipodia structures in a migrating breast-cancer-derived cell line and colocalizes with a known S100A4-interacting protein, heavy chain IIA (MHC-IIA) at the leading edge. We also demonstrate that S100A4 mutants that are either defective in their ability to dimerize or calcium-bind are unable to interact with MHC-IIA. An S100A4 mutant that is deficient for calcium binding retains the ability to form homodimers, suggesting that S100A4 can exist as calcium-free or calcium-bound dimers in vivo. However, a calcium-bound S100A4

monomer only interacts with another calcium-bound monomer and not with an S100A4 mutant that does not bind calcium. Interestingly, despite the calcium dependence for interaction with known protein partners, calcium binding is not necessary for localization to lamellipodia. Both wild type and a mutant that is deficient for calcium binding colocalize with known markers of actively forming leading edges of lamellipodia, Arp3 and N-WASP. These data suggest that S100A4 localizes to the leading edge in a calcium-independent manner, and identification of the proteins that are involved in localizing S100A4 to the lamellipodial structures will provide novel insight into the mechanism by which S100A4 participates in metastasis.

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HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano O. Cuvier A. Losada
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M. Hirano

The long-term goal of our research program is to understand the molecular mechanisms of chromosome segregation, an essential process for maintaining genome stability during mitosis in eukaryotes. Our current efforts are focused on studying two multiprotein complexes, cohesin and condensin, that have central roles in sister chromatid cohesion and condensation, respectively. At the heart of the two complexes lie members of a large family of chromosomal ATPases, the structural maintenance of chromosome (SMC) family. SMC proteins are also conserved in bacterial and archaeal species, providing us with an excellent opportunity to address the evolutionarily conserved, fundamental aspects of chromosome mechanics. We take multidisciplinary approaches including biochemistry, cell biology, genetics, and biophysics.

Distinct Contributions of Condensins I and II to Mitotic Chromosome Assembly and Segregation

T. Ono, A. Losada, M. Hirano, T. Hirano [in collaboration with M.P. Myers and A.F. Neuwald, Cold Spring Harbor Laboratory]

Accumulating lines of evidence demonstrate that condensin is one of the key regulators in mitotic chromosome assembly and segregation. The canonical condensin complex (henceforth condensin I) was originally identified in our laboratory as a major chromosomal component required for the establishment and maintenance of mitotic chromosomes in *Xenopus laevis* egg extracts. Subsequent studies in a number of model organisms showed that the structure and function of this complex are highly conserved from yeast to humans. During the past year, we have discovered a second class of condensin (termed condensin II) from HeLa cells and *Xenopus* egg extracts. Condensins I and II share the same pair of SMC core subunits (SMC2 and SMC4), but they contain different sets of non-SMC subunits (HEAT and kleisin; Table 1). Condensin-II-specific subunits are widespread in animals and plants but are not found in yeast. Intriguingly, however, condensin II apparently substitutes for condensin I in the nematode *Caenorhabditis elegans*.

To address how the two complexes contribute to the establishment of mitotic chromosome architecture, we have used a combination of two powerful functional assays, small interfering RNA (siRNA)-mediated depletion in HeLa cells, and immunodepletion from *Xenopus* egg extracts. Depletion of condensin I- or II-specific subunits in HeLa cells causes a distinct, highly characteristic defect in chromosome morphology, i.e., "swollen" or "curly" chromosomes, respectively. In the absence of both complexes, mitotic chromosomes display a very fuzzy appearance in which sister chromatids are no longer discernible. Not surprisingly, such morphological defects result in a number of abnormalities in chromosome align-

TABLE 1 Distribution of condensin subunits in model organisms

Subunits	Complexes	*H. sapiens*	*X. laevis*	*A. thaliana*	*D. melanogaster*	*C. elegans*	*S. pombe*	*S. cerevisiae*
SMC2	I, II	hCAP-E	XCAP-E	AtCAP-E1&-E2	DmSMC2	MIX-1	Cut14	Smc2
SMC4	I, II	hCAP-C	XCAP-C	AtCAP-C	DmSMC4/gluon	SMC-4	Cut3	Smc4
HEAT	I	hCAP-D2	XCAP-D2	CAB72176	CG1911	–	Cnd1	Ycs4
HEAT	I	hCAP-G	XCAP-G	BAB08309	CG17054	–	Cnd3	Ycs5/Ycg1
Kleisin γ	I	hCAP-H	XCAP-H	AAC25941	Barren	–	Cnd2	Brn1
HEAT	II	hCAP-D3	XCAP-D3	At4g15890.1	CG31989	HCP-6	–	–
HEAT	II	hCAP-G2	XCAP-G2	At1g64960.1	?	F55C5.4	–	–
Kleisin β	II	hCAP-H2	XCAP-H2	At3g16730.1	CG14685	C29E4.2	–	–

ment and segregation during mitosis. In *Xenopus* egg extracts, condensin I has a predominant role (i.e., no individual chromosomes are assembled in its absence), whereas condensin II has a minor yet important role in shaping the chromosomes. In the absence of condensin II, individual chromosomes are assembled, but they display an abnormal morphology, reminiscent of the curly chromosomes observed in human cells depleted of condensin II. Correspondingly, condensin I is more abundant than condensin II in the egg extracts, whereas the two complexes are equally abundant in HeLa cells. Immunofluorescence studies reveal that condensins I and II show distinct distributions along the axis of chromosomes assembled both in vivo (in HeLa cells) and in vitro (in *Xenopus* egg extracts). We speculate that the relative abundance of the two complexes could act as a key determinant of the size, shape, and physical properties of mitotic chromosomes in different organisms or at different stages of development.

Role of BimD Proteins in the Regulation of Sister Chromatid Cohesion

A. Losada, T. Hirano

Cohesin is a multisubunit complex that constitutes the molecular "glue" between the sister chromatids. Given its fundamental importance, the temporal and spatial association of cohesin with chromatin during the cell cycle must be tightly regulated. One candidate for proteins that regulate cohesin is BimD/Pds5, which was originally identified by genetic screens in yeast and other fungi. To study the function of this class of proteins in *Xenopus* egg extracts, we screened a *Xenopus* cDNA library and found that this organism possesses two BimD-like proteins (termed XBimD1 and XBimD2) encoded by two different genes. Preliminary data suggest that XBimD1 and XBimD2 display distinct chromatin-binding properties. XBimD1 binds to both interphase and mitotic chromatin with similar efficiency, in the presence or absence of cohesin. In contrast, XBimD2 targets interphase chromatin in a cohesin-dependent manner and, like cohesin, most of it dissociates during mitotic prophase. Depletion of XBimD1 or XBimD2, or both, has little effect on the association of cohesin with chromatin during interphase, but partially compromises cohesin's dissociation at the onset of mitosis. No clear defects in cohesion or condensation are observed in mitotic chromosomes assembled under this condition. To better understand the function of BimD proteins, we have also prepared antibodies against their human orthologs. In nuclear extracts prepared from HeLa cells, most hBimD1 and hBimD2 appear to be physically associated with cohesin. This robust interaction may reflect a more intimate cooperation between cohesin and BimD proteins in somatic cells than in embryonic extracts. RNA interference experiments are under way to gain additional insights into this possibility.

Scc2 is Required for the Establishment of Sister Chromatid Cohesion in *Xenopus* Egg Extracts

P. Gillespie, T. Hirano

The establishment of sister chromatid cohesion involves at least two separable steps. The first is the binding of cohesin to chromatin prior to the initiation of DNA replication, and the second is the construction of a cohesin-mediated linkage during or immediately after DNA replication. Previous genetic studies in yeast suggested that a conserved protein, Scc2, is required for the loading of cohesin onto chromatin in vivo. To gain mechanistic insights into the function of Scc2 by using *Xenopus* egg cell-free extracts, we first cloned cDNAs corresponding to the *Xenopus* ortholog of Scc2. Our sequence analysis shows that *Xenopus* have two distinct Scc2 isotypes, XScc2α (245 kD) and XScc2β (260 kD). The two isotypes, which are also present in humans, are distinguished by their different carboxyl termini that arise by alternate splicing of a single gene. We raised a set of antibodies that specifically recognize XScc2α or XScc2β or both, and used them for immunodepletion experiments. We find that simultaneous depletion of XScc2α and XScc2β from an egg extract inhibits the association of cohesin with chromatin, although individual depletion of each isotype has only a limited effect. Thus, at least one of the XScc2 isotypes is required for the efficient loading of cohesin onto chromatin in the extract. Salt extraction experiments suggest that once cohesin is loaded onto chromatin, XScc2 proteins are no longer required to maintain the

cohesin-chromatin interaction. Finally, and most importantly, we find that metaphase chromosomes assembled in the absence of XScc2 show a gross defect in chromatid pairing, a phenotype indistinguishable from that observed in cohesin-depleted extracts. These results indicate that Scc2 has a direct role in the establishment of sister chromatid cohesion in higher eukaryotes.

Regulation of a Bacterial SMC Protein Complex

M. Hirano, T. Hirano

The genome of the gram-positive bacterium *Bacillus subtilis* encodes a single SMC gene whose disruption causes multiple phenotypes including decondensation and missegregation of chromosomes. Its gene product, BsSMC, is a homodimer that adopts a V-shaped structure with an ATP-binding catalytic domain at each end. Recent studies involving genetics and bioinformatics suggested that two small proteins, called ScpA and ScpB, might function together with BsSMC in vivo. During the past year, we have purified ScpA and ScpB and have shown that they associate with the catalytic domains of BsSMC in an ordered fashion in vitro. When combined with the "transition state" mutant of BsSMC, ScpA promotes stable engagement of two catalytic domains in an ATP-dependent manner. In solution, this occurs intramolecularly, thereby closing the DNA-transporting gate of an SMC dimer. ScpB further stabilizes this conformation and prevents BsSMC from binding to double-stranded DNA (dsDNA). In contrast, when the mutant BsSMC is first allowed to interact with dsDNA, subsequent addition of ATP and ScpA leads to assembly of large nucleoprotein complexes, possibly by facilitating intermolecular engagement of catalytic domains from different SMC dimers. Our results show that ATP, ScpA, and ScpB cooperate to modulate the engagement/disengagement cycle of BsSMC and have both positive and negative roles in regulating its interaction with DNA. These findings have broad implications in our understanding of how the dynamic behavior of condensin and cohesin might be regulated in eukaryotic cells.

DNA Nano-manipulation to Probe the Action of Condensin

T. Hirano [in collaboration with T. Strick, Cold Spring Harbor Laboratory]

In collaboration with Terence Strick's group here at CSHL, we have used a DNA nano-manipulation technique to monitor in real-time the interactions between a single DNA molecule and the condensin complex (condensin I). In these experiments, a linear DNA molecule is tethered at one end to a glass surface and at the other end to a small magnetic bead. Permanent magnets are used to pull on and rotate the bead, allowing us to quantitatively control the mechanical constraints applied to the DNA. The three-dimensional position of the magnetic bead is determined using video-microscopy and real-time particle tracking algorithms, allowing us to determine the end-to-end extension of the DNA. By monitoring the DNA extension as a function of time in the presence of condensin I purified from *Xenopus* egg mitotic extracts, we are now starting to catch a glimpse of how condensin utilizes the energy of ATP hydrolysis to "condense" dsDNA in a highly dynamic fashion.

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EPITHELIAL CELL BIOLOGY AND CANCER

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EARLY EVENTS IN BREAST CANCER

Disruption of epithelial architecture and loss of growth control are thought to be early events in cancer. Nevertheless, how oncogenes coordinately deregulate cell architecture and growth control to initiate transformation of epithelial cells in vivo is not known.

In epithelial cells lining glandular structures (e.g., breast, prostate, pancreas, thyroid, endometrium, and cervix), proteins and cell-cell junction complexes are distributed asymmetrically in the apical-basal axis, a property referred to as epithelial cell polarity. Various aspects of epithelial polarity are lost early duing carcinogenesis. Moreover, highly metastatic carcinomas display a complete loss of apical-basal polarity. Identification of the mechanisms that govern loss of epithelial architecture will provide us with important insights into the steps that regulate carcinoma initiation and progression and will lead to identification of novel targets for diagnosis and treatment of early-stage carcinomas.

Loss of proliferation control is a critical step in cancer. Normal breast epithelial cells, present in acinar structures in vivo, have low proliferation rates, whereas epithelial cells present in hyperplastic lesions or carcinoma of the breast have higher proliferation rates, suggesting that proliferation control is lost early in carcinoma. Normal mammary epithelial cells (MEC) grown in three-dimensional (3-D) matrices form acini-like structures with low proliferation rates as observed in a resting normal breast tissue. In contrast, breast-tumor-derived cells neither form acini-like structures nor undergo proliferation arrest when placed under 3-D culture conditions, suggesting that the mechanisms that regulate cell cycle control during 3-D morphogenesis are lost during transformation.

The ErbB family of oncogenic receptor tyrosine kinases contains four members, namely, ErbB1 (epidermal growth factor receptor/human epidermal growth factor receptor [HER-1]), ErbB2 (HER-2/Neu), ErbB3, and ErbB4. ErbB2 overexpression and/or amplification has been implicated in a number of epithelial cancers including that of the breast, ovary, and prostate. Amplification of ErbB2 is observed in 20–30% of breast cancers and is correlated with poor clinical prognosis in node-positive patients. Herceptin, an antibody targeted against ErbB2, is used as a therapeutic agent in adjuvant settings for patients who have breast cancers that express high levels of ErbB2. Despite the compelling evidence for the role of ErbB family in breast cancer, it has been challenging to decipher the signaling and biological specificities of ErbB receptor activation because ligand binding induces both homo- and heterodimerization among the ErbB receptors, thus resulting in combinatorial interactions and complicating our ability to determine how different ErbB dimers transform mammary epithelia.

We have developed a method to control dimerization and activation of ErbB receptors without contribution from endogenous receptors; we have also modified a cell culture approach where nontransformed MECs form 3-D acini-like structures containing a single layer of polarized growth-arrested epithelial cells when grown within a matrix rich in laminin and collagen (Matrigel). By combining the controlled dimerization strategy and the 3-D cell culture system, we have recently shown that inducible activation of ErbB2, in growth-arrested 3-D organized mammary epithelial acini, reinitiates proliferation, disrupts epithelial cell polarity, and induces formation of noninvasive multiacinar structures that share several properties with early-stage lesions in vivo. We plan to identify the mechanisms by which oncogenes such as ErbB2 transform polarized, 3-D organized, epithelial cells.

How Does ErbB2 Reinitiate Proliferation in Growth-arrested 3-D Acini?

B. Xiang

The mechanism by which ErbB2 promotes G_1 progression has received much attention. Yet, our understanding of the molecular mechanism by which ErbB2 promotes cell cycle in epithelial cells is not clear.

Overexpression of ErbB2 in human mammary epithelial cells or tumor-derived epithelial cells lines induces G_1 progression by inducing down-regulation or mislocalization of the Cip/Kip family of cyclin-dependent kinase inhibitor (CKI) p27 and/or by up-regulating the levels of the G_1 cylcin and cyclin D1 and by activation of cyclin-dependent kinase 2 (CDK2). Studies using mouse models suggest that cyclin D1 has a critical role in ErbB2-induce mammary tumorgeneisis.

In primary breast tumors, cyclin D1 overexpression correlates with estrogen-receptor (ER)-positive status and good clinical prognosis whereas, overexpression of cyclin E1 and cyclin E2 correlates with ER-negative status and poor clinical prognosis, suggesting that E-type cyclins may have a more critical role in aggressive breast cancers. Since ErbB2 overexpression correlates with ER-negative status and poor clinical prognosis, the conclusions derived from mouse model studies are confusing.

We are investigating how mammary epithelial cells undergo proliferation arrest during morphogenesis in the 3-D matrix, and how activation of ErbB2 reinitiates proliferation. It is likely that inducible activation of ErbB2 in 3-D acini will provide novel insights into the mechanisms by which the cell cycle is regulated during tumorigenesis.

How Is Epithelial Cell Polarity Lost during Transformation?

T. Haire, C.-M. Chen

The early stages of epithelial cell-derived cancers (carcinoma) are characterized by abnormal cellular proliferation and loss of tissue organization, resulting in multilayered or filled ductal structures referred to as hyperplasia or ductal carcinoma in situ. The epithelial cells within the multilayered lesions are surrounded by cell-cell interactions and possess no apical surface or apical polarity. Many oncogenes that are implicated in the development of carcinomas induce gross morphological changes when overexpressed in epithelial cells in vitro. For instance, we and others have shown that activation of ErbB2, Ki-Ras Raf, Fos, Jun, Rho and Rac, CDC42, and v-Src disrupts polarity of epithelial cells in culture. Although loss of epithelial cell polarity and tissue organization are common events in carcinoma, very little is known about the mechanisms that regulate these events.

In normal polarized epithelia, the boundary between the apical and basolateral membranes is defined by the presence of at least three classes of transmembrane proteins, namely, occludins, claudins, and junctional adhesion molecules (JAMs), collectively referred to as the apical junction complex (AJC). The mechanism by which AJCs are positioned during polarization of epithelial cells involves a molecular complex containing the mammalian homolog of *Caenorhabditis elegans* partitioning-defective 3 (mPar3)/mPar6/atypical protein kinase C/CDC42 or Rac (referred to as the Par complex). Consistent with this view, formation of AJC is affected by overexpression of mPar6, expression of inactive forms of aPKC, or expression of active versions of CDC42 or Rac. Although the Par protein complex is critical for establishment of cell polarity, it is not known whether it is affected by oncogenic changes that disrupt polarity during cancer progression.

We have previously established that activation of ErbB2 induces disruption of apical-basal polarity in epithelial cells, we are now investigating the mechanisms by which oncogenes such as ErbB2 disrupt epithelial cell polarity.

Role of the Rho Family of GTPases in ErbB2-induced Transformation of 3-D Epithelial Acini

M. Moore

The Rho family of GTPases are known to regulate the cytoarchitecture of epithelial cells in culture by affecting the actin cytoskeleton, cell-cell junction proteins such as cadherins, and apical-basal polarity. Although the Rho family of proteins are only weak oncogenes themselves, they are thought to have an important role by cooperating with other oncogenes. Overexpression of Rho family of proteins are thought to regulate each of the multiple steps during tumorigenesis, such as proliferation, loss of cell architecture, and cell invasion. In fact, Rho family members are overexpressed in many epithelial tumors including that of breast, colon, and pancreas, suggesting that they may have a role in transformation of epithelial cells.

Since activation of ErbB2 regulates cell proliferation and apical-basal polarity of epithelial cells, we are investigating the role of the Rho family of GTPases in ErbB2-induced transformation of polarized epithelial cells.

How Does ErbB2 Induce Disruption of Epithelial Cell Polarity and Reinitiation of Proliferation?

A. Lucs

We have previously shown that activation of ErbB2 induces reinitiation of cell cycle progression and loss of epithelial cell polarity as monitored by expression of a proliferation-associated antigen ki-67, and localization of the tight junction-associated protein, ZO-1. To understand how ErbB2 reinitiates proliferation in polarized epithelial cells, we have mutated the five autophosphorylation sites present in the cytoplasmic tail of ErbB2 that are known to have important roles in receptor function. We have generated ten mutant variants of ErbB2, five of which have four of five tyrosine residues mutated, leaving one residue as wild-type, and five variants that have one of five tyrosine residues mutated, leaving four tyrosines wild type. We plan to use these mutants to investigate how ErbB2 regulates reinitiation of proliferation and determine whether a relationship exists between ErbB2's ability to affect proliferation and its ability to disrupt epithelial cell polarity.

Do ErbB Homodimers and Heterodimers Differentially Regulate Epithelial Cell Polarity?

C.-M. Chen, B. Xiang

Several ErbB ligands such as EGF, TGFα, Amphiregulin, NRG1, and Cripto and receptors ErbB1, ErbB2, and ErbB3 have been shown to be overexpressed in a number of carcinomas, including those of the breast and prostate. Expression of high levels of ErbB2 is thought to favor homodimeric interactions among ErbB2 receptors. However, ErbB2/ErbB3 heterodimers have been shown to be more aggressive in their transforming ability, and more recent observations suggest that ErbB2 and ErbB3 are co-overexpressed in breast cancer, suggesting a strong role for the ErbB2/ErbB3 heterodimer.

It is not known whether ErbB dimers differ in their ability to transform polarized, proliferation-arrested epithelial cells. We have modified our dimerization strategy such that we can induce formation of heterodimers between two members of the ErbB receptor family using small-molecule ligands. We have generated cell lines where we can induce either homodimers or heterodimers within a single cell by chosing different types of dimerizing ligands. We plan to investigate whether receptors dimers differentially regulate epithelial cell polarity and proliferation.

Generation of Rapid Mouse Models to Study Oncogene-Tumor Suppressor Gene Interactions in the Mammary Gland

A. Rosenberg

Mouse models expressing oncogenes in genetic backgrounds that lack tumor suppressor genes will serve as valuable tools to study tumor initiation, progression, and treatment. Although there are several studies highlighting the importance of genetic interactions in mouse mammary epithelium, it is well appreciated that both strain variations and promoters used for overexpression complicate the interpretation of these analyses. For instance, accelerated occurrence of salivary tumors in a mouse mammary tumor virus (MMTV) LTR-*ras*/*p53*$^{-/-}$ genetic background impaired the analyses of mammary tumors. Moreover, promoters used to express oncogenes (MMTV or whey acidic protein [WAP]) may not target all subtypes of mammary epithelia (stem cell and nonstem cells) and hence resulted in cell bias for tumor initiation. We propose to generate a system that will offer three major advantages over the existing systems: (1) use of an ubiquitous promoter to express oncogenes in all cell types of mammary epithelium (stem cell and nonstem cell compartments), (2) use of the shRNA to down-regulate expression of tumor suppressor genes and hence allow analyses of hypomorphic alleles, and (3) use of a mammary gland reconstitution approach to analyze genetic interactions between oncogenes and tumor suppressor genes for a fraction of the cost and time.

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CELL BIOLOGY OF THE NUCLEUS

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Most cellular processes can trace their beginnings to the nucleus where a gene is activated resulting in the production of an RNA molecule which must get processed and transported to the cytoplasm. Although many genes are constitutively expressed, a large number are up-regulated and down-regulated at various times during the cell cycle and development. *Trans*-acting factors have the daunting task of locating and gaining access to specific genes within the three-dimensional nuclear space. Altering the transcriptional status of a particular gene involves a series of events, including chromatin remodeling and the recruitment of a large number of factors involved in either transcription/RNA processing/export or silencing events. However, a failure of the correct set of factors to associate with their target substrates at the correct time will result in changes in the gene expression program which can lead to a disease phenotype. Although much biochemical information is available regarding some of the factors involved in these processes, the spatial and temporal aspects of the regulation of gene expression/silencing and the dynamics of the nuclear domains that they occupy are less well understood. During the past year, we have focused a significant amount of our efforts on two main areas: (1) characterizing chromatin structure as a gene transits from an inactive heterochromatic state to an active euchromatic state, and (2) characterization of the distribution and expression of SR pre-mRNA splicing factors in the plant *Arabidopsis thaliana*.

VISUALIZING GENE EXPRESSION IN LIVING CELLS

We have previously developed a mammalian cell line that allows us to directly visualize a stably integrated inducible genetic locus as well as its RNA and protein products in living cells. The locus is based on a 20-kb plasmid that contains at its 5′-end *lac* operator repeats that when bound by a cyan fluorescent protein (CFP)-*lac* repressor fusion protein allows the integration site to be visualized. A tetracycline-responsive element enables the minimal cytomegalovirus (CMV) promoter to be regulated. Transcription by RNA polymerase II (pol II) results in an RNA containing stem loop structures that are specifically recognized by the bacteriophage MS2-binding protein. Expression of MS2-binding protein fused to yellow fluorescent protein (YFP) containing a nuclear localization signal allows us to see the RNA as it is being transcribed. The RNA encodes CFP with a peroxisome-targeting signal to allow the protein product of this transcription unit to be visualized as it concentrates in cytoplasmic peroxisomes. Using this system, we have observed the dynamics of proteins associated with inactive (i.e., HP1, Suv39h1) as well as active (i.e., VP-16, RNA pol II) chromatin.

As YFP-HP1α was dynamically depleted from the locus during the induction of transcription and we could not detect the histone H3 tri-MeK9 modification at transcriptionally active loci (2.5 hr postinduction), we were interested in understanding how this modification is removed from the chromatin during transcriptional activation. As a histone demethylase has not yet been identified and the histone H3 variant, H3.3, has been shown to be deposited into active ribosomal DNA in a replication-independent manner (Ahmad and Henikoff *Mol. Cell 9:* 1191 [2002]), we were interested in determining whether histone exchange may be a general mechanism through which a heterochromatic region of chromatin is transformed into the active state.

Before the induction of transcription, H3.3-YFP did not show any specific deposition at the locus. However, a small spot of H3.3-YFP was first observed approximately 7.5 minutes after the induction of transcription adjacent to the region marked by CFP-*lac* repressor. Significant incorporation of H3.3-YFP appeared around the periphery of the locus and subsequently progressed into the interior. Over time, H3.3-YFP deposition became highly concentrated at the locus and did not completely overlap with the regions marked by CFP-*lac* repressor, suggesting that deposition may specifically occur in the regions associated with the transcription machinery. As significant depo-

sition of H3.3 at the locus is not seen until later time points (75–150 min postinduction), it is possible that histone exchange is a late event in the transition of heterochromatin to the active state, perhaps having more of a role in changing and maintaining the epigenetic state of a gene than in aiding in the progression of transcription. It is also possible that in order for transcription to begin, histone exchange must first occur in specific sequence elements, such as promoters; we are not able to detect deposition in such small regions. Subsequently, histones may be exchanged throughout the entire coding region resulting in the significant accumulation visualized at later time points. Nucleoli were also labeled by H3.3-YFP, as confirmed by double labeling with an antibody to fibrillarin, which is consistent with the deposition of H3.3 into rDNA. Therefore, histone exchange appears to be part of the mechanism by which heterochromatin can be converted to active chromatin.

DYNAMIC ORGANIZATION AND TISSUE-SPECIFIC EXPRESSION OF SR SPLICING FACTORS IN *ARABIDOPSIS*

The organization of the pre-mRNA splicing machinery has been extensively studied in cultured mammalian and yeast cells and far less is known about its dynamics in living plant cells and in different cell types of an intact organism. During the past year, we examined the expression, organization, and dynamics of pre-mRNA splicing factors (SR33, SR1/atSRp34, and atSRp30) under control of their endogenous promoters in *Arabidopsis thaliana*. Distinct tissue-specific expression patterns were observed, and in addition, differences in the distribution of the respective proteins within the nuclei of different cell types were identified. These differences may be a reflection of the organization of chromatin and the amount of interchromatin space within the respective cell types. In addition, the metabolic state of the cells, including the distribution of active genes and their transcriptional levels, may also affect the distribution of SR proteins. As a higher metabolic level correlates with more active chromatin and/or more transcription sites, a larger amount of SR proteins will therefore be recruited to these transcription sites from speckles, resulting in a lower ratio of splicing factors in speckles to that in the diffuse nucleoplasmic population. Future studies will directly examine the relationship of the compaction state of chromatin to the organization of the pre-mRNA splicing machinery in different *Arabidopsis* cell types.

Leaf epidermal pavement cell nuclei, which have an obvious speckled pattern of pre-mRNA splicing factors, were used to visualize the dynamics of splicing factors in vivo by time-lapse three-dimensional microscopy. A region of rosette leaf (~0.5 cm^2) was cut and mounted in water, and a 60x/1.20 N.A. water immersion objective lens was used to follow the dynamics of nuclear speckles at room temperature. Data sets were collected at 15-second intervals over a 30-minute time period. During periods of up to 30 minutes, most speckles were observed to be moving within a constrained volume of ~1 μm^3 with respect to the total nuclear volume of ~280 μm^3. Some speckles were observed to fuse or bud from each other during the imaging period. A few speckles moved into another nearby area and then resumed their constrained movement. Other speckles disassembled into the nucleoplasm or formed at new sites. Some adjacent speckles were connected by less intensely labeled fluorescent signals. The SR splicing factors appeared to dissociate/associate with speckles or to exchange between nearby speckles, resulting in changes in the shape of these speckles. In about 5% of the leaf epidermal pavement cells, 1–3 speckles entered into the nucleoli, and moved rapidly within this nuclear domain. Identical dynamic events of speckles were observed in *SR1/atSRp34-YFP, SR33-YFP*, and *atSRp30-YFP* transgenic plants. The dynamic organization of plant speckles was found to be closely related to the transcriptional activity of the cells and could also be modulated by the phosphorylation state of the splicing factors.

To further determine the dynamics of SR proteins in speckles, we used fluorescence recovery after photobleaching (FRAP) analysis. A region of leaf (~0.5 cm^2) from *SR1/atSRp34-YFP* or *SR33-YFP* transgenic plants was mounted in water. After five single scans were acquired, a 1.7 x 1.7 μm^2 nuclear region that contains a speckle was photobleached, and a series of images were acquired immediately after bleaching. Subsequently, the relative intensity of fluorescence within the photobleached area was calculated. In addition, the level and rate of the fluorescence recovery, as fluorescent molecules from outside the photobleached zone migrated into the bleached area, were determined. Consistent results were obtained from leaves of plants containing the transgene *SR1/atSRp34-YFP* or *SR33-YFP*; the SR proteins exchange from the speckle with a half time of recovery of approximately 2.5 seconds. The organization and dynamic behavior of speckles in *Arabidopsis* cell nuclei provide significant

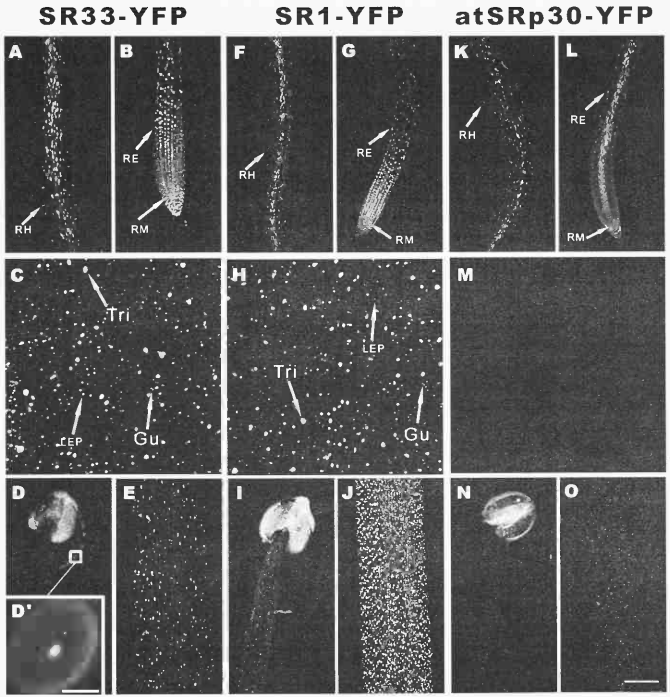

FIGURE 1 Expression patterns of SR33-YFP, SR1/atSRp34-YFP, and atSRp30-YFP in *Arabidopsis*. Projections of a series of confocal optical sections of influorescence stems, anthers, roots and leaves are shown. (*A*, *F*, *K*) Basal section of primary root; (*B*, *G*, *L*) tip section of primary root; (*C*, *H*, *M*) leaf; (*D*) anther; (*D'*) enlarged view of a pollen grain; (*E*, *J*, *O*) inflorescence stem. Different cell types are highlighted with arrows: (Tri) trichomes; (Gu) guard cells; (LEP) large (C) and small (H) leaf epidermal pavement cells; (RH) root hairs; (RM) root meristematic cells; (RE) root epidermal cells. Bar, 100 μm; (inset *D'*) 10 μm.

insight into understanding the functional compartmentalization of the nucleus and its relationship to chromatin organization within various cell types of a single organism.

In addition to observing SR protein dynamics, we observed differences in SR protein expression levels in different cell types, tissues, and developmental stages (Fig. 1). For example, in the vascular bundle of the primary root tip section, atSRp30 is highly expressed, and SR33 and SR1/atSRp34 are expressed at significantly lower levels. In contrast, SR33 is expressed throughout the root meristem, elongation zone, and specialization zone, whereas SR1 expression drops off significantly in the specialization zone. In leaves, we observed high expression of SR33 and SR1/atSRp34 and minimal expression levels of atSRp30; in pollen grains, all three proteins were expressed at similar levels. At later flowering stages, SR1/atSRp34 expression decreased to a lower level than SR33. Finally, in the inflorescence stems, SR1 is expressed at extremely high levels throughout the stem, whereas SR33 and atSRp30 are expressed at

progressively lower levels. In *pSR33:SR33-YFP* transgenic plants, we observed fluorescence in nearly all tissues or organs and at all developmental stages. This implies that SR33, a SC35-like splicing factor, is active in the processing of most pre-mRNAs in *Arabidopsis*, whereas atSRp30, a tissue-specific SF2/ASF-like splicing factor, may be involved in generating tissue-specific transcripts within cells of the vascular system. In contrast, the high level of expression of SR1 in the inflorescence stems and leaves may be indicative of a more global role in constitutive pre-mRNA splicing, as well as a potential role in alternative pre-mRNA splicing. Further studies are necessary to elucidate the roles of specific splicing factors in regulating different aspects of plant development.

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MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

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The papillomaviruses are a group of viruses that infect and transform the basal epithelium inducing proliferation of the cells at the site of infection. The resulting tumors (warts) are in most cases benign and will usually regress after some time, but certain types of human papillomaviruses (HPVs) give rise to tumors that are prone to progress toward malignancy, especially frequently cervical carcinoma. Indeed, HPV infection appears to be a necessary cause of invasive cervical carcinoma and thus represents one of the few firmly established links between viral infections and the development of cancer.

An impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for analysis of the viral life cycle. These viruses normally require specialized differentiating cells that only with difficulty can be generated in cell culture. However, for a bovine papillomavirus (BPV-1), a convenient cell culture system exists where viral gene expression, oncogenic transformation, and viral DNA replication can be studied. Thus, BPV has become a useful model for these aspects of the viral life cycle. The DNA replication properties of the papillomaviruses show some unique and interesting characteristics. As part of their normal life cycle, these viruses can exist in a state of latency, which is characterized by maintenance of the viral DNA as a multicopy plasmid in infected cells. The copy number of the viral DNA is tightly controlled, and the viral DNA is stably inherited under these conditions. Papillomaviruses therefore provide a unique opportunity to study plasmid replication in mammalian cells. In addition, the viral DNA replication machinery represents one of the most promising targets for antiviral therapy.

In previous years, we have reported the characterization of the papillomavirus replicon and the identification of the viral components that are required for viral DNA replication. In recent years, we have directed our attention toward the biochemical events that are associated with initiation of DNA replication. We are studying the biochemical properties of the viral E1 and E2 proteins and how these two proteins interact with the viral origin of DNA replication and with the cellular replication machinery to generate initiation complexes. Our studies demonstrate that the E1 protein has all the characteristics of an initiator protein, including ori recognition, DNA-dependent ATPase activity, and DNA helicase activity. The transcription factor E2, whose precise function has remained more elusive, appears to serve largely as a loading factor for E1. Through direct physical interactions with both E1 and the ori, E2 provides sequence specificity for the formation of the initiation complex.

We are currently attempting to elucidate how the E1 and E2 proteins orchestrate the precise biochemical events that precede initiation of DNA replication at the viral ori. These events include binding of the initiator to the ori, the initial opening of the DNA duplex, as well as the assembly and loading of the E1 replicative helicase at the replication fork. Our studies so far indicate that these activities are generated in an ordered process that involves the sequential assembly of E1 molecules on the ori. This sequential assembly generates different complexes with different properties that in turn recognize ori, destabilize the double helix, and function as the replicative DNA helicase.

GENERATION OF SEQUENCE SPECIFICITY FOR INITIATOR DNA BINDING

Viral initiators of DNA replication are dependent on the ability to find and recognize the viral origin of DNA replication in a vast excess of host-cell DNA in the infected cell. Interestingly, many viral initiators appear to bind DNA with modest to low selectivity, raising the question of how the ori is recognized. For the papillomaviruses, the E2 protein contributes to highly selective DNA binding by binding cooperatively with E1 to the ori. We, as well as others, have believed that the role of E2 is to recruit E1 to the ori. Interestingly, we have recently determined that the E2 protein functions by an entirely different molecular mechanism. By analyzing the DNA-binding properties of the E1 protein, we found that although the full-length E1 protein binds DNA with low specificity, the DNA-binding domain (DBD) of E1 binds with high specificity. The explanation for this apparent paradox

is that two different DNA-binding activities are present in the full-length E1 protein. The E1 DBD is capable of highly sequence-specific DNA binding, but a different DNA-binding activity, present in the helicase domain of the E1 protein, binds DNA nonspecifically. The net result of the presence of both a nonspecific and a specific DNA-binding activity is that full-length E1 binds DNA with low specificity. We now also understand how E2 acts to provide high specificity DNA binding. E2 interacts with the E1 helicase domain and prevents this domain from contacting DNA. As a result, in the presence of E2, E1 binds DNA only via its DBD, resulting in highly specific DNA binding. As E2 is displaced, the E1 helicase domain is free to engage the sequences flanking the E1-binding sites, generating a complex with intrinsically low sequence specificity, but bound to a specific site. The reason for this complex arrangement is clearly that the nonspecific DNA-binding activity which resides in the E1 helicase domain has an important role in later events where the E1 protein melts and unwinds the ori DNA. This raises the interesting possibility that initiator proteins in general may employ similar procedures for generation of highly sequence-specific DNA binding. Furthermore, this mode of E1 binding provides us with a simple model for how larger initiator complexes are assembled on the ori. By addition of E1 dimers in an ordered fashion, the final form, a double hexamer of E1, can be assembled without rearrangement of the individual subunits.

MUTATIONAL ANALYSIS OF THE E1 DBD SURFACE

The DBD of the E1 protein clearly is responsible for the highly site-specific DNA binding that the E1 protein is capable of, which in turn is responsible for recognition of the origin of DNA replication in the viral genome. Furthermore, on the basis of both structural and biochemical studies of the E1 DBD, the DBD also provides the dimerization surface for the E1 protein and also contains an interaction surface for the DBD from the E2 protein. In addition, based on imaging analysis of, for example, SV40 T antigen, it is likely that the E1 DBD takes an active part in formation of large complexes such as the double hexamer, which appear to be the entity that melts and unwinds the origin of DNA replication. To identify additional functions in the DBD, we have performed a complete surface mutagenesis of the E1 DBD. Based on the high-resolution structure of the E1 DBD, we have substituted approximately 70 surface residues for alanine, avoiding areas known from the structural analysis to be involved in DNA binding and dimerization. After screening these E1 mutants for expression and for in vivo DNA replication activity, we were able to identify 18 mutants with defects in DNA replication. Six of these were defective for interaction with E2, whereas the remaining 12 mutants showed unimpaired interaction with E2 and consequently were defective for DNA replication for other reasons. To determine what biochemical functions might be defective in these mutants, we expressed and purified the 12 mutant E1 proteins and tested them in five different in vitro assays related to DNA replication. None of the mutants showed a defect in either the ATPase or the nonspecific DNA helicase activities, indicating that the protein structure was not greatly affected by the mutations and that most likely, the ability to form hexameric structures is unaffected by the mutations. However, all 12 mutants had severe defects for in vitro DNA replication, as well as defects in melting activity and in fragment unwinding activity. These results indicate that the ability to form double hexamers might be impaired since both melting and unwinding activities require formation of the double hexamer.

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DNA REPLICATION AND CHROMATIN INHERITANCE

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The research focuses on three aspects of the inheritance of genetic material from one cell generation to the next. DNA in chromosomes replicates during S phase in eukaryotic cells and concomitant with DNA synthesis is the assembly of nucleosomes onto the DNA and duplication of epigenetically determined chromatin structures. We have been studying the mechanism of DNA replication and how this is linked to cell cycle control, chromatin inheritance and chromosome segregation during mitosis.

ROLE OF ORC IN THE INITIATION OF DNA REPLICATION IN MAMMALIAN CELLS

The origin recognition complex (ORC) is a six-subunit protein complex that was initially identified from the yeast *Saccharomyces cerevisiae*, but it has orthologs in all eukaryotes and even in archaeal species. ORC binds to origins of DNA replication and in yeast is a stable six-subunit protein complex. On the other hand, ORC composition in human cells is dynamic, with the largest subunit being degraded as cells enter into S phase. We have also shown that the bulk of the Orc6 subunit is primarily not associated with the other ORC subunits, but it is associated with centromeres and the cytokinesis furrow during mitosis and cytokinesis. Disruption of Orc6 using siRNA (short interfering RNA) methods causes cells to either stop DNA replication or fail to execute cytokinesis, depending on where in the cell cycle the lack of Orc6 had its effect.

In the last year, we have investigated one of the other subunits of the human ORC, the Orc2 subunit. Using multiple antibodies directed against this protein, we have shown that Orc2 localizes at multiple locations in interphase cells, including small punctate foci that most likely correspond to sites of DNA replication and to larger heterochromatic foci that also contain the heterochromatin protein HP1. Both of these structures are present in cells in the G_1 phase of the cell cycle, but as cells enter and progress thorough S phase, Orc2 is lost from both of these structures, even though HP1 still remains associated with heterochromatin. During prophase as cells enter mitosis, Orc2 localizes to centric heterochromatin. Interestingly, we also find a fraction of Orc2 associated with centrosomes at all stages of the cell division cycle, including interphase **and** all stages of mitosis.

The function of Orc2 was investigated by depleting the protein from various human cell lines using RNA interference (RNAi) genetics. Cells lacking Orc2 arrested in the cell cycle at two separate stages, probably depending on when during the cell cycle the Orc2 was depleted. About 70% of the cells arrested with a block to DNA replication and remained attached to the culture dish. In these cells, all HP1 localization to heterochromatic foci was disrupted. On the other hand, about 30% of the cells arrested with a rounded mitotic-like appearance, and in these cells, chromosomes were abnormal and the mircotubule spindle was disorganized, with many cells having multiple centrosomes. The chromosomes in such cells were not properly condensed, and they did not attach to the disorganized mitotic spindle. Such observations suggest that Orc2, like the Orc6 subunit, has a role in chromosome structure and segregation, in addition to the expected role in initiation of DNA replication. We are following up these interesting studies to examine how mitosis is disrupted and why such cells do not complete chromosome segregation.

CLAMP LOADERS: DNA REPLICATION AND DAMAGE CONTROL

In previous years, we have characterized the proteins from human cells that are essential for replication of simian virus 40 (SV40) DNA in the presence of the virus-encoded T antigen. One of the proteins we found is a five-subunit protein complex called RFC (replication factor C) that is an ATP-dependent clamp loader. RFC loads the DNA polymerase clamp proliferating cell nuclear antigen (PCNA) onto DNA to initiate DNA synthesis at origins of DNA replication and at the start of every Okazaki fragment. RFC contains

four small subunits that are shared with other protein complexes in the cell, including one that contains the Rad17 protein. The new complex has been designated RSR (Rad17-RFC2–5). In last year's Annual Report, we described the purification of RSR and the demonstration that it can load another clamp onto DNA. The new clamp is a heterotrimer containing the Rad1, Hus1, and Rad9 proteins that form a complex called RHR. The RSR loads the RHR clamp onto DNA in a ATP hydrolysis-dependent manner much like RFC loads the polymerase clamp PCNA.

Mutations in the yeast genes encoding Rad17 and the genes encoding the RHR proteins show phenotypes of increased sensitivity to DNA-damaging agents and a lack of checkpoint control of the cell division cycle that arrests cell division when chromosomes are damaged in interphase. Thus, it was likely that the RHR clamp did not function like the PCNA polymerase clamp. We further investigated the mechanism of RSR loading RHR onto DNA and made the unexpected finding that RHR is loaded onto DNA that contains a recessed 5′ end at a junction between double-stranded and single-stranded DNA (see Fig. 1). In contrast, our previous studies have demonstrated that RFC loads PCNA onto 3′-recessed ends of DNA, consistent with its role in recruiting a DNA polymerase to the 3′ primer for processive DNA replication. The observation that RHR loads on to a 5′-recessed end of DNA is not consistent with RHR acting as a DNA polymerase clamp. Rather, we suggest that the recessed 5′ ends are generated during the process of repair of damage in the DNA and at telomeres during elongation of telomere repeats. We also demonstrated that the loading requires the single-stranded DNA-binding protein RPA (replication factor A), suggesting that RPA has to be associated with single-stranded DNA before the RSR can act to load RHR onto DNA. Such a scenario implies that the RSR-RHR machinery functions after DNA damage recognition and not as an initial sensor of DNA damage, as was previously thought.

CHROMATIN ASSEMBLY DURING DNA REPLICATION

As the DNA replication forks copy DNA to produce the two sister chromatids for each chromosome, the DNA must be reassembled into a nucleosome-containing chromatin structure. The parental nucleosomes are disrupted during DNA replication, but the epigenetic modifications that they contain must be inherited so

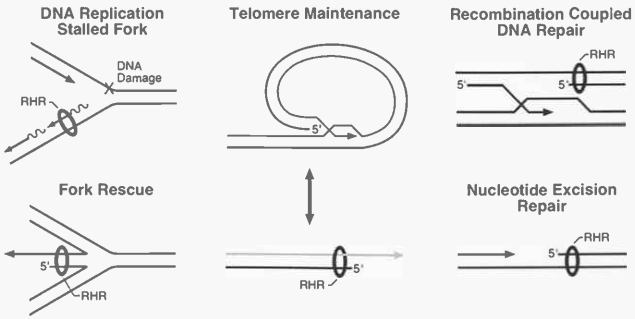

FIGURE 1 Possible substrates onto which the checkpoint clamp loader RSR may load its clamp (RHR) DNA maintenance pathways, including those depicted here, generate intermediates containing free and/or recessed 3′ ends that are processed by a variety of proteins. These structures also contain recessed 5′ ends, whose fate in these reactions is unclear. Given that RSR loads RHR (depicted as a ring or donut encircling the DNA) onto recessed 5′ ends in vitro, recessed 5′ ends generated in vivo in the depicted pathways can be considered potential substrates. They all contain adjacent single-stranded DNA that could be bound by RPA. RHR has been shown to be required for checkpoint signaling in response to DNA replication fork arrest, double-stranded breaks, and improper telomere maintenance. The RHR clamp is proposed to protect the recessed 5′ end from extensive degradation by exonucleases and to promote resolution of these structure back to duplex DNA. (Reprinted from Ellison V. and Stillman B. 2003. *PLoS Biol.* **1:** 231–243.)

that gene expression states from one cell generation to the next can be remembered. One protein that participates in the inheritance of nucleosomes is the chromatin assembly factor-1 (CAF-1), a three-subunit protein that binds histones and can assemble them onto DNA after passage of a DNA replication fork. CAF-1 is tethered to the DNA replication fork by interaction with PCNA.

The *S. cerevisiae* CAF-1 is not essential, but cells lacking this protein are sensitive to DNA damaging agents, probably reflecting the fact that chromatin assembly must also occur on repaired DNA and CAF-1 is known to couple DNA repair and nucleosome assembly in vitro. Yeast is unusual in that it lacks substantial amounts of heterochromatin found in cells from metazoan species and only contains one form of histone H3, the form associated with transcribed DNA in mammalian cells. Mammalian cells contain an additional form of histone H3 that is primarily associated with nontranscribed DNA, including heterochromatin. We therefore tested if CAF-1 is essential for cell cycle progression in human cells, to determine if DNA replication and nucleosome assembly are coupled, as we originally found during SV40 DNA replication in vitro.

Expression of the largest subunit of CAF-1 was targeted for silencing by selective siRNA treatment. When depleted from human cells grown in culture, the cells arrested either in the G_1 phase or in early S phase, with very little DNA synthesis occurring. A Chk2-mediated (but not Chk1-mediated) DNA damage checkpoint was triggered in these cells, which was unexpected since Chk1 is thought to be the intra-S-phase checkpoint that monitors DNA damage. The activation of the Chk2 protein kinase suggests that chromatin assembly is directly linked to later events that regulate entry into mitosis. Eventually, however, the CAF-1-depleted cells died of apoptosis. Thus, in human cells, CAF-1 is essential and is necessary for coupling chromatin assembly to DNA replication. We are further investigating the mechanism of this coupling.

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes including cell proliferation and differentiation. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects the activity of the kinases that phosphorylate it and the protein phosphatases that catalyze the dephosphorylation reaction. We study the expanding family of protein tyrosine phosphatases (PTPs), which, like the kinases, comprise both transmembrane, receptor-linked forms and nontransmembrane, cytoplasmic species and represent a major family of signaling enzymes. We are utilizing a variety of strategies to characterize the physiological function of members of the PTP family. Disruption of normal patterns of tyrosine phosphorylation has been implicated in several human diseases. Therefore, insights into the mechanisms involved in modulating PTP function may ultimately yield important information to help counter such diseases.

During the last year, there have been several changes in laboratory personnel. Toshi Fukada left to take up a faculty position at the RIKEN Research Center for Allergy and Immunology at Osaka University; T.C. Meng took up a faculty position at the Institute of Biological Chemistry, Academica Sinica, Taiwan; and Helena Palka accepted a position on the Research Faculty in the Department of Biochemistry and Molecular Genetics, University of Illinois, Chicago. We have been joined by Naira Gorovits, who conducted her Ph.D. research in Maureen Charron's lab at Albert Einstein College of Medicine; and Zhong Yao, who obtained his Ph.D. working with Dr. Rony Seger at The Weizmann Institute of Science, Israel. Most recently, Antonella Piccini joined the lab after completing postdoctoral studies with Robert Malinow here at CSHL. Finally, Regina Hecker worked in the lab during the summer on an intership during which she conducted research for a Diploma Thesis from the University of Applied Sciences Oldenburg, Emden, Germany.

PTP: A THERAPEUTIC TARGET FOR TREATMENT OF DIABETES AND OBESITY

It is now apparent that the protein tyrosine phosphatase (PTP1B) is a major regulator of signaling events induced by insulin and leptin. In fact, PTP1B is now recognized in the pharmaceutical industry as an important target for development of novel strategies for therapeutic intervention in diabetes and obesity. The active site of members of the PTP family is highly charged, consistent with its recognition of a charged, phosphorylated residue as substrate. This, and the fact that the essential Cys residue at the active site is prone to oxidation, has presented challenges to the design of therapeutic inhibitors, suggesting that alternative methods for manipulating the activity of PTP1B may be of benefit.

We have identified two elements that are important in the regulation of transcription from the PTP1B promoter. Expression of PTP1B is induced by the p210BcrAbl oncoprotein, a PTK (protein tyrosine kinase) that is responsible for the initial manifestations of chronic myologenous leukemia. We identified a p210BcrAbl-responsive sequence (PRS) in the PTP1B promoter and showed that is recognized by Egr-1 and Sp C_2H_2 zinc-finger transcription factors, which act in a reciprocal manner to regulate expression of PTP1B in response to p210BcrAbl. We have identified an enhancer sequence upstream of the PRS which functions as a binding site for Y-box-binding protein-1 (YB-1) and have shown that the levels of PTP1B could be manipulated by altering the levels of YB-1. Depletion of YB-1, by expression of a specific antisense construct, led to an approximately 70% decrease in expression of PTP1B, without alteration of the levels of TCPTP (the closest relative of PTP1B), and resulted in increased sensitivity to insulin and enhanced signaling through the cytokine receptor gp130. Nevertheless, attempts to manipulate insulin signaling and the levels of blood glucose in animals, by administration of antisense oligonucleotides direct-

ed against YB-1, proved unsuccessful, with complications most likely arising from the known pleiotropic actions of YB-1.

REGULATION OF PTP FUNCTION BY OXIDATION

We have also investigated the regulation of PTP1B function by reversible oxidation. In general terms, the signature motif [I/V]HCXXGXXR[S/T], which defines the PTP family of enzymes, contains an invariant Cys residue, which functions as a nucleophile in catalysis. Due to the unique environment of the PTP active site, this Cys residue displays an unusually low pK_a, which enhances its nucleophilic properties but renders it susceptible to oxidation. Once oxidized, the active-site Cys can no longer function as a nucleophile. A wide variety of physiological stimuli that result in stimulation of tyrosine phosphorylation are also accompanied by the production of reactive oxygen species (ROS), which is required for an optimal tyrosine phosphorylation response. Work from several labs has demonstrated that PTPs are an important target of ROS in this context. The operating principle is that the stimulus (hormone, growth factor, etc.) enhances tyrosine phosphorylation directly, by activation of a PTK, and/or indirectly, by inactivation of a PTP. Therefore, one function of ROS produced following agonist stimulation is to inactivate transiently the critical PTP that provides the inhibitory constraint upon the system, thus facilitating the initiation of the signaling response to that stimulus.

We have demonstrated that stimulation of Rat1 fibroblasts with insulin led to the production of ROS, in particular H_2O_2. Expression of catalase inhibited both tyrosine phosphorylation of the insulin receptor (IR) and downstream signaling to PKB/Akt, thus highlighting the importance of ROS production for optimal signaling. Using a modified in-gel phosphatase assay we have developed, we showed that two PTPs were rapidly and reversibly oxidized in response to insulin, and we have identified these as PTP1B and TC45. A role for PTP1B in the regulation of IR signaling is now well established, validating our strategy of using reversible oxidation as a method to tag those PTPs that are oxidized in response to a defined stimulus, thereby revealing those PTPs that may be critical for down-regulating the signaling response to that stimulus. We have now investigated the oxidation of PTP1B from a structural perspective, as part of a long-standing collaboration with David Barford (ICR, London, U.K.). For reversible oxidation to represent a mechanism to regulate PTP function, it is essential that the active-site Cys residue is not oxidized further than the sulfenic acid (–SOH) form. Higher oxidation to sulfinic acid (SO_2H) or sulfonic acid (SO_3H) is an irreversible modification that would be inconsistent with a regulatory mechanism. We incubated crystals of PTP1B with stoichiometric quantities of H_2O_2 in order to determine the structure of the oxidized, sulfenic acid form of the enzyme. Surprisingly, we never detected the sulfenic acid; instead, a sulfenyl-amide species was observed, in which a covalent bond was formed between the sulfur atom of the active-site Cys and the main-chain nitrogen of the adjacent residue, Ser-216, leading to formation of a novel 5-atom ring structure at the active site. The consequences for the architecture of the active site are profound (Fig. 1). The PTP loop, containing the signature motif, and Tyr-46, from the pTyr loop, which are normally buried in the structure, flip out of the active site to adopt solvent exposed positions. It is important to note that these effects are readily reversible upon incubation of the crystals with reducing agent. This novel oxidation-dependent post-translational modification, which also occurs in solution, disrupts substrate recognition by PTP1B and renders Tyr-46 susceptible to phosphorylation, possibly explaining some of the reports in the literature of tyrosine phosphorylation of members of the PTP family. Furthermore, the conformational change in PTP1B that accompanies sulfenyl-amide bond formation exposes the oxidized Cys to reducing agents, thereby facilitating reduction to the active form of the enzyme. Therefore, the discovery of this novel sulfenyl-amide-modified PTP1B reveals a mechanism by which generation of higher oxidized forms of the active-site Cys is prevented and reduction back to the active state is facilitated. It will be interesting to ascertain whether this represents a general mechanism for the regulation of PTP function by reversible oxidation. If so, stabilization of the oxidized, inactive form of PTPs, such as PTP1B, by small-molecule drugs, may offer a novel strategy for therapeutic intervention in diseases associated with aberrant PTP activity.

We have also investigated the role of TC45 in the regulation of insulin signaling. Ablation of TC45 expression by RNA interference in Rat1 fibroblasts and HepG2 cells led to hyperphosphorylation of the insulin receptor and stimulation of insulin-induced activation of PKB/Akt. In addition, in collaboration with Tony Tiganis (Monash University, Australia), we observed that insulin-induced phosphorylation of the insulin receptor and activation of PKB/Akt were enhanced and sustained in immortalized mouse

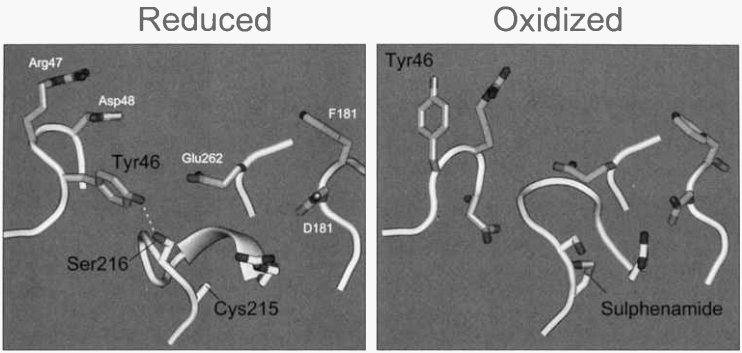

FIGURE 1 Oxidation of PTP1B is accompanied by profound conformational changes at the active site. Incubation of PTP1B with stoichiometric amounts of H_2O_2 generated an unexpected covalent modification at the active site. Upon oxidation to sulfenic acid, nucleophilic attack by the backbone N atom of Ser-216 on the Sγ atom of Cys-215 generates a cyclic sulfenamide, which is a puckered five-membered ring. As a result, two critical H-bonds, which are responsible for the integrity of the active site, are broken, resulting in profound conformational changes in which the active site springs open, resulting in inhibition of substrate binding. The signature motif which normally forms the base of the active-site cleft loses its helical conformation. In this motif, Gly-218 shifts by 7 Å and, most importantly, Cys-215 now becomes exposed to the environment, thereby facilitating reduction back to the active state. In addition, Tyr-46, which is normally buried in the active site, where it defines the depth of the cleft and determines specificity for tyrosyl residues in substrates, now becomes exposed. In this conformation, Tyr-46 is susceptible to phosphorylation which may introduce a further tier of control over the regulation of members of the PTP family. It is important to stress that although these conformational changes are dramatic, they are nonetheless reversible. This modification suggests a mechanism for redox regulation of the PTPs that will facilitate fine-tuning of tyrosine phosphorylation-dependent signaling pathways.

embryo fibroblasts (MEFs) generated from TCPTP$^{-/-}$ mice compared to control TCPTP$^{+/+}$ animals. Therefore, this study, which was initiated by the observation of transient insulin-induced oxidation of TC45, has illustrated that, in addition to PTP1B, TC45 is a negative regulator of signaling in response to insulin.

RECEPTOR-LIKE PROTEIN TYROSINE PHOSPHATASES

We identified a receptor PTP termed DEP-1 (density enhanced PTP-1), the expression of which is increased in cells in culture approach confluence, suggesting a role as an inhibitor of cell proliferation. DEP-1 has recently been identified by the lab of Peter Demant as the product of a tumor suppressor gene that is mutated in colon, lung, and breast cancers. Using substrate-trapping mutant forms of DEP-1, we have identified two physiologically important substrates: p120 catenin and the hepatocyte growth factor/scatter factor receptor Met. p120 catenin, which was originally identified as a substrate of the protein tyrosine kinase Src, regulates cadherin-based cell adhesion. In addition to the cadherin-associated pool of p120, there is also a cytoplasmic pool of the protein that has been implicated in the regulation of Rho, Rac, and Cdc42 small GTP-binding proteins. The distribution between these pools is regulated by cell confluence, with the bulk of p120 catenin found at cell-cell junctions in confluent cultures, and also potentially by phosphorylation of p120 catenin. In the case of Met, ligand-induced tyrosine phosphorylation of particular sites within the PTK underlies the regulation of mitogenic, motogenic, or morphogenic responses. We observed that DEP-1 preferentially recognized signaling sites in Met, including the Gab1-binding site (Y1344) and a carboxy-terminal site implicated in morphogenesis (Y1365), whereas tyrosine residues in the activation loop of the kinase (Y1230, 1234, 1235) were not preferred targets of the PTP. The ability of DEP-1 preferentially to dephosphorylate particular tyrosine residues that are required for Met-induced signaling suggests that DEP-1 may function in controlling the

specificity of signals induced by this PTK, rather than as a simple "off-switch" to counteract PTK activity. This functional interaction between Met and DEP-1 raises the possibility that up-regulation of Met may be coupled with down-regulation of DEP-1 in the progression of certain human tumors. We are currently exploring the signaling function of DEP-1 in cell lines that express the phosphatase in an inducible manner. We have also been studying a distinct PTP, termed PTPμ, which is expressed primarily in endothelial cells and is also increased in expression following the formation of cell-cell contacts. This receptor PTP was the first for which a ligand was identified, with our demonstration that it participates in homophilic binding interactions, i.e., a molecule of PTPμ on one cell binds to another PTPμ molecule on an adjacent cell. The immunoglobulin domain in the extracellular segment is critical for this binding function. Our most recent analyses have highlighted the importance of the homophilic binding site regulating the level and subcellular location of PTPμ, thereby controlling the function of the enzyme and the maintenance of normal cell physiology.

THE DUAL SPECIFICITY PHOSPHATASES (DSPs)

The PTP family of enzymes, which are defined by the presence of the signature sequence (I/VHCxxGxxR [S/T]), can be divided into two broad categories, the Tyr-specific or classical PTPs and the dual specificity phosphatases (DSPs), which have the capacity to dephosphorylate Ser/Thr and Tyr residues on proteins, in addition to nonprotein substrates such as inositol phospholipids. An obvious major difference between the enzymes in these two categories is the design of the active site. In the classical PTPs, the active site is present as a deep cleft on the surface of the protein, the depth of which is defined by a conserved Tyr residue that restricts specificity only to phosphotyrosine residues. In the DSPs, this Tyr residue is absent and the active-site cleft is shallower, allowing it to accommodate the shorter side chains of phospho-Ser and phospho-Thr. The first DSP to be described was the enzyme VH1, which is essential for infectivity of the virions of *Vaccinia* virus. In mammals, the DSPs have been implicated in the regulation of various fundamental physiological functions, including proliferation, differentiation, survival, and transition through the cell cycle. We have searched the completed human genome sequence to identify the complement of DSPs. This proved to be a greater challenge than our similar analysis of the classical PTPs because, although they contain the active-site signature motif, the DSPs are less well conserved overall and are therefore harder to identify on the basis of sequence than the pTyr-specific members of the PTP family. We identified a minimal DSP catalytic domain, and our search yielded 43 DSPs that are related to VH1 and contain this domain, several of which are currently uncharacterized. Each of these DSP genes was also found in mouse. These VH1-like DSPs can be divided into three classes on the basis of structural similarity. Within each class, subcategories have also been delineated, each of which is represented in *Drosophila* and most were observed in *Caenorhabditis elegans*.

We have begun to explore the structure, regulation, and function of several of these DSPs. Our studies have revealed two related enzymes, Jsp1 and DSP18/Jsp2, that may underlie a novel tier of control of the Jnk family of MAP kinases, which are activated in response to pro-inflammatory cytokines and environmental stresses and are implicated in the regulation of proliferation and apoptosis. We have identified two novel DSPs that appear to be more highly expressed in tumor tissue and cell lines than normal. Such overexpression may indicate a direct link between the DSP and the etiology of the disease. Finally, we have identified a locus from which two distinct, but closely related, DSPs are expressed from alternative reading frames of a single gene. This gene organization is conserved in the mouse. The situation is reminiscent of that reported for the p16Ink4/p19Arf locus; however, unlike that example in which two structurally and functionally distinct proteins are produced from the same gene, the gene we have identified yields two closely related members of the same enzyme family. These DSPs, which we term DSP7 and DSP17, display highly restricted tissue expression patterns and are expressed predominantly late in development in adult tissues. Their functions are currently under investigation.

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Shanta Hinton

RAS AND RHO GTPases AND THE CONTROL OF SIGNAL TRANSDUCTION

L. Van Aelst B. Boettner, R. Packer, E.-E. Govek, M. Uchida, J. Janas, V. Velamoor, S. Newey, M. Zhao

Research in my laboratory focuses on signal transduction pathways involving members of the Ras and Rho GTPases and the physiological processes that they regulate. Ras and Rho family members encode low-molecular-weight guanine nucleotide-binding proteins that function as binary switches by cycling between an active GTP-bound state and an inactive GDP-bound state. It is only in their GTP-bound state that they are able to interact with downstream effector molecules, which mediate their effects. The ratio of the two forms is regulated by the opposing effects of guanine nucleotide exchange factors (GEFs), which promote the exchange of bound GDP for GTP, and the GTPase-activating proteins (GAPs), which stimulate hydrolysis of bound GTP. Alterations that affect normal Ras and Rho function have been found to result in the development of several disease processes including cancer, as well as inflammatory and neuropathological disorders. To understand how alterations in Ras and Rho signaling components contribute to these disease processes, it is essential to comprehend their normal functions both at a molecular and at a cellular level. Efforts in my laboratory have focused on defining the role and mechanisms by which Ras and Rho family members exert their effects on specific aspects of tumorigenesis and on neuronal development.

During the past year, we have made significant progress toward understanding the molecular basis by which the Ras-related GTPase, Rap1, exerts its effects on epithelial morphogenesis and how the RasGAP-associated protein, $p62^{dok}$, affects cell proliferation. Furthermore, we have provided additional insights into the role of Ras and Rho GTPases in neuronal development and function. Specifically, we have uncovered a role for the X-linked mental retardation protein, oligophrenin-1, in dendritic spine development.

THE AF-6 HOMOLOG CANOE ACTS AS A RAP1 EFFECTOR DURING DORSAL CLOSURE OF THE *DROSOPHILA* EMBRYO

Our studies in *Drosophila* and in mammalian systems support a role for Rap1 proteins in the control of epithelial cell-shape changes, coordinated movements of epithelial cells, and cell/extracellular matrix (ECM) adhesion. These are pivotal events governing tumor progression and wound healing processes; yet the signaling pathways mediating these effects of Rap1 remain unknown. Previously, we identified the mammalian junctional protein AF-6 as a Rap1-interacting protein. However, the functional analyses of mammalian Rap1 and AF-6 proteins have been hampered by the fact that these proteins are largely refractory to loss-of-function and/or gain-of-function analysis. Hence, we decided to gain insights into their functions using *Drosophila*, a genetically more tractable model system (in collaboration with Dr. U. Gaul, of The Rockefeller University). The morphogenetic dorsal closure (DC) process in *Drosophila*, which relies on elongation and migration of epithelial cells, has proven to be an excellent model for the study of the molecular mechanisms regulating epithelial cell-shape changes.

We found that both DRap1 and Canoe (the *Drosophila* orthologs of Rap1 and AF-6) are essential in the DC process and provide evidence that Canoe acts as a downstream effector of DRap1 in this developmental step. Specifically, we found that Canoe binds to the activated form of DRap1 in a yeast two-hybrid assay and that the two molecules colocalize to the adherens junction. We also observed that *DRap1* and *canoe* mutants display very similar phenotypes in DC. Finally, our genetic interaction experiments showed that DRap1 and Canoe act in the same molecular pathway during DC, with DRap1 acting upstream of Canoe, and that the function of both molecules depends on their ability to interact. Taken together, these experiments demonstrate that Canoe is a "bona fide" effector of DRap1 in the context of DC. Interestingly, we also obtained evidence that Canoe has a dual function during DC. The first function is controlled by DRap1 and is independent of JNK signaling, whereas the second function is not activated by DRap1 but feeds into JNK signaling. We have initiated screens to identify additional Canoe-interacting proteins to obtain further insights into Canoe's mode

of action in dorsal closure. To complement these studies, we will also assess a role for the mammalian counterparts of these *Drosophila* proteins in mammary epithelial polarity and invasive behavior. The information gained from our studies in *Drosophila* will facilitate the design of experiments addressing the role of the mammalian orthologs in epithelial cells.

p62^{dok} FUNCTIONS AS A NEGATIVE REGULATOR OF MITOGENIC AND ONCOGENIC p210$^{bcr-abl}$ SIGNALING

p62^{dok} is a protein initially identified as a 62-kD constitutively tyrosine-phosporylated RasGAP-associated protein, in p210$^{bcr-abl}$-expressing cells. This protein was termed dok (*d*ownstream *o*f *k*inases), since it was also found to be a common substrate of many receptor and cytoplasmic tyrosine kinases. Subsequently, four additional dok family members have been identified. Among them, p62^{dok} and dok-2 share in common the ability to bind to RasGAP. To investigate the role of p62^{dok}, we utilized different cell types derived from p62^{dok} null mice, generated by P.P. Pandolfi (Memorial Sloan-Kettering Cancer Center, New York). In collaboration with Pandolfi's group, we obtained evidence that p62^{dok} acts as a negative regulator of growth-factor-induced cell proliferation. We observed that p62^{dok}-deficient cells possess a significantly higher proliferation rate in response to growth factors and that this increase in cell proliferation can be suppressed by ectopic expression of p62^{dok}. Our subsequent studies demonstrated that p62^{dok} exerts its negative effect on growth-factor-induced cell proliferation at least in part by negatively influencing the Ras/MAPK (mitogen-activated protein kinase) pathway. Moreover, for p62^{dok} to act as a negative regulator of the Ras/MAPK pathway, we have shown that p62^{dok} must be recruited to the membrane. The growth-factor-triggered translocation of p62^{dok} to the plasma membrane involves the activation of phosphatidylinositol-3 kinase (PI3-kinase) and binding of its plekstrin-homology (PH) domain to 3′-phosphorylated phosphoinositides.

Using p210$^{bcr-abl}$ mouse model systems, we observed that p62^{dok} inactivation causes a significant shortening of the latency of the fatal myeloproliferative disease induced by p210$^{bcr-abl}$. We also found that ectopic expression of p62^{dok} in Mo7 myeloid cells markedly decreases the growth potential of these cells. These findings implicate a role for p62^{dok} in the negative regulation of p210$^{bcr-abl}$ signaling and leukemogenesis. How p62^{dok} opposes the leukemogenic potential of p210$^{bcr/abl}$ remains to be defined. We are currently undertaking proteomic and genetic approaches to address this question. Notably, we observed that p62^{dok} null mice do not show gross aberrant phenotypes and display normal steady-state hemopoiesis. This may be due to the fact that other dok family members can compensate for the loss of p62^{dok} function. In collaboration with Pandolfi's group, we are currently analyzing the phenotypes of p62^{dok} and dok-2 double knockout mice.

RAS AND RHO GTPases REGULATE KEY ASPECTS OF NEURONAL FUNCTION AND DEVELOPMENT

Accumulating data demonstrate that members of the Ras and Rho GTPases control important aspects of neuronal development and function. This is further highlighted by the findings that several diseases causing cognitive impairment (including neurofibromatosis, autism, and mental retardation) are associated with mutations in members of the Ras and Rho GTPases or in the molecules (GEFs and GAPs) that control their activity. A major challenge remains to unravel how these molecules affect neuronal development and function and how perturbing their activities can cause cognitive deficits.

In collaboration with Drs. J. Zhu and R. Malinow here at CSHL, we have investigated the role of Ras and Rap1 in activity-dependent synaptic plasticity, which is believed to underlie key aspects of brain development, learning, and memory. Recent studies have revealed that AMPA (α-amino-hydroxy-5-methyl-4-isoazole) receptor (AMPA-R) trafficking is important in synaptic plasticity; however, the signaling pathways regulating this trafficking are poorly understood. We found that Ras mediates activity-induced synaptic enhancement by driving synaptic delivery of AMPA-Rs containing long cytoplasmic tails requiring p42/p44 MAPK activation. In contrast, we observed that Rap1 mediates activity-induced synaptic depression by removing synaptic AMPA-Rs containing short cytoplasmic tails requiring p38 MAPK activation. Taken together, these studies indicate that Ras and Rap1 serve as independent regulators for potentiating and depressing central synapses. We are currently investigating the contribution of the immediate downstream effectors of the Ras and Rap GTPases to AMPA-R trafficking.

My lab has also embarked on the functional characterization of *oligophrenin-1*, which encodes a Rho-GAP, and whose loss of function is associated with nonsyndromic or nonspecific X-linked mental retardation (MRX). MRX is characterized by mental

impairment without any other distinctive clinical features. Eight genes involved in MRX have been cloned to date, and importantly, three of these genes encode regulators or effectors of the Rho GTPases. This has led to the hypothesis that abnormal Rho GTPase signaling may be a prominent cause of MRX, although it has been unclear how mutations in these Rho-linked genes lead to MRX since studies on the effects of loss of these genes on neuronal development have not been reported. We found that oligophrenin-1 is required for normal dendritic spine morphogenesis. Using RNA interference (RNAi) and antisense RNA approaches, we demonstrated that down-regulation of oligophrenin-1 levels in CA1 hippocampal neurons in organotypic slices resulted in a significant decrease in dendritic spine length. Importantly, spine morphological changes of the same magnitude have been reported for a mouse model of Fragile X, indicating that such changes can compromise synaptic plasticity.

We further obtained evidence that the RhoA/Rho-kinase signaling pathway mediates the action of oligophrenin-1 knockdown on dendritic spine length. First, the effect of oligophrenin-1 knockdown on spine length most closely mimics the phenotype observed upon expression of a constitutively active RhoA mutant, rather than an activated Rac1 or Cdc42 mutant. Second, the effect of oligophrenin-1 knockdown on spine length can be rescued by inhibition of Rho kinase, a major downstream target of RhoA. Treatment of *oligophrenin-1* small interfering RNA (siRNA)-transfected hippocampal slices with the Rho kinase inhibitor Y-27632 resulted in a significant increase in spine length in CA1 neurons compared to *oligophrenin-1* siRNA-transfected neurons in nontreated slices. Notably, Y-27632 treatment of hippocampal slices transfected with control duplex siRNA did not significantly increase spine length compared to untreated slices. This observation suggests that the RhoA/Rho-kinase pathway is repressed under physiological conditions, which is consistent with studies by Luo and colleagues. Our findings that full-length oligophrenin-1 did not increase spine length further supports this repression model, since overexpression of a negative regulator of a primarily inactive pathway would not be expected to result in a detectable phenotype. Taken together, our data support a model in which oligophrenin-1 normally acts to repress the RhoA/Rho kinase pathway to maintain spine length. Upon removal of oligophrenin-1, inhibition of RhoA is relieved, resulting in activation of Rho kinase and a concomitant decrease in spine length (see Fig. 1). More recently, we have identified an interaction between

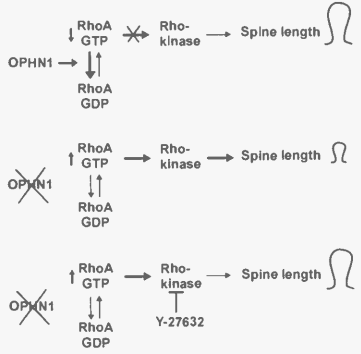

FIGURE 1 Model of the pathway through which knock down of oligophrenin-1 levels results in a reduction in spine length. Under normal physiological conditions in CA1 neurons in hippocampal slices, RhoA activity, and as a result Rho-kinase activity, is repressed in the presence of oligophrenin-1 in order to maintain normal spine length (*top panel*). Knockdown of oligophrenin-1 relieves negative regulation of oligophrenin-1 on RhoA activity, causing an increase in RhoA and Rho-kinase activities, and ultimately a reduction in spine length (*middle panel*). When this pathway is interfered with in cells with reduced oligophrenin-1 levels by using Y-27632 to inhibit Rho kinase, the reduction in spine length caused by the decrease in oligophrenin-1 levels can be rescued (*bottom panel*).

oligophrenin-1 and Homer, placing oligophrenin-1 within a postsynaptic complex that potentially links oligophrenin-1 to glutamate receptor signaling. Our findings provide insights into how mutations in a Rho-linked MRX gene may compromise neuronal function and demonstrate that RNAi is a valuable tool for studying gene function in hippocampal slices.

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BIOINFORMATICS AND GENOMICS

Computers have an indispensable role in the rapidly progressing arena of biomedical research. CSHL scientists working in the allied disciplines of genomics and bioinformatics use computer science and mathematics to explore the molecular basis of life from many angles. By developing sophisticated software and novel databases, these scientists are creating tools that are vital for interpreting the volumes of information that are emerging with increasing speed from many studies of genes, genomes, proteins, and proteomes.

Lincoln Stein's lab uses computer science to give meaning to the tremendously large data sets that are resulting from a variety of genome research projects. One of several databases they have developed allows researchers to compare the genomes of rice, wheat, and corn and to identify genes that might be used to engineer hardier, more productive varieties. Another, still a work in progress, is a searchable database of fundamental processes in human biology called Genome KnowledgeBase. Stein's lab, in collaboration with researchers from around the world, also published a study this year that details their plans to map the regions of common genetic variability in the human genome by comparing three major world populations. The resulting map will cut cost and effort in the search for genes that indicate susceptibility to cancer and other disorders with genetic components.

W. Richard McCombie's lab continues to contribute significantly to several genome sequencing projects. This year, McCombie's lab collaborated with U.S. colleagues to sequence chromosome 10 of the world's most important food source, rice. The scientists are continuing work to finish sequences of parts of three other rice chromosomes. In collaboration with the Martienssen lab, they continued to develop a new technique ("methylation filtration") for capturing gene-rich sequences of the corn genome, proving it to be a superior method for finding new genes. They continued collaborating with the Martienssen, Zhang, and Hannon labs to identify the positions of genes within plant and animal genomes, including the mouse genome, which McCombie's lab recently helped sequence.

Bud Mishra's lab is bringing mathematics and computer science to bear on a challenging new field known as systems biology. One of his lab's projects is to create a unified theoretical framework within which scientists can understand life's biochemical pathways and the evolutionary processes that mold them. These researchers merge math and biology to develop models of how genomes evolve and to explore how evolution generates particular networks and modules of interacting components that function as biochemical pathways.

Michael Zhang's lab has created software for identifying signature sequences within DNA that reveal the presence of important genetic elements such as promoters (DNA segments that control gene activity) or exons (DNA segments likely to code for proteins). They have compared the human and mouse genomes and created a database of closely related DNA sequences shared by humans and mice. They are currently using newly developed tools to compare differences in specific pieces of DNA between prostate cancer and normal cells, in a method that can be applied to other diseases, improving our understanding of cancer and generating possible targets for treatment.

Andrew Neuwald's lab has applied the power of statistical analysis to the prediction of protein structure and function. These researchers have developed a technique for identifying the most distinctive molecular features of any particular family of proteins. Once identified among closely and moderately related proteins, such features can be used to track down the most distant relatives of a protein family. As a result, the scientists can infer the properties of poorly characterized proteins by assigning them to protein families with known functions. Such analysis has revealed previously unrecognized roles for certain proteins in human disease.

GENOME SEQUENCE ANALYSIS

W.R. McCombie V. Balija, M. de la Bastide, N. Dedhia, S. Dike, M. Katari, F. Katzenberger, B. Miller, S. Muller, L. Nascimento, A. O'Shaughnessy, L. Palmer, L. Spiegel, M. Yu, T. Zutavern

We have been working to develop strategies and technologies to extract biological information from plant and animal genomes. The problems in the plant genome are surprisingly different from those in the animal genome. One of the major challenges in plant genomics is to extract sequence information from the genes out of a background of repeat sequences, which can comprise 80–90% of the genome. The genes themselves are quite compact in plants, but there are a large variety of genomes that are important to sequence. This is complicated by recent observations that even different strains of plant species can have very different genome structures. As a result, efforts have been directed at developing methods to select, sequence, and order the genes of large plant genomes while bypassing the nongene regions. These strategies are critically important to compare the sequences of model genomes. We have made considerable progress in these areas as described below.

In the animal world, there are several mammalian genome sequences available. We have been focusing our efforts on the experimental determination of gene structure in the mouse. In particular, we have been focusing on the determination of transcriptional start sites and alternate first exons in mouse genes.

Rice Genome Sequencing

W.R. McCombie, V. Balija, M. de la Bastide, N. Dedhia, S. Dike, M. Katari, F. Katzenberger, B. Miller, S. Muller, L. Nascimento, A. O'Shaughnessy, L. Palmer, L. Spiegel, T. Zutavern

We have continued our efforts as part of the International Rice Genome Sequencing Consortium to sequence the genome of rice. In collaboration with Rod Wing's lab at the University of Arizona, we completed our regions of chromosome 10 in 2002. In 2003, we finished our regions of chromosome 3 ahead of schedule. We were funded in 2003 to continue sequencing by finishing regions of chromosomes 5, 9, and 11. We sequenced almost 5 Mb of rice in 2003.

In addition to continuing the sequencing of rice, we carried out an analysis of the whole of chromosome 10 which we finished sequencing last year. Working with collaborators who sequenced other areas of chromosome 10, we published an analysis of this chromosome in 2003.

Sequencing and Analysis of Gene Rich Regions of the Maize Genome

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In collaboration with Rob Martienssen's lab here at CSHL, we have previously raised the possibility of selectively enriching whole-genome clone libraries for genes and developed methods to do this (Rabinowicz et al. 2003). Subsequently, we and others have been working to carry out a large-scale test of these strategies, as well as to develop additional methods and strategies to use these data as a starting point in the generation of a set of sequences corresponding to the maize genes, which have been ordered, oriented, and linked to the physical map of maize.

We sequenced about 100,000 sequence reads from methyl-filtered libraries which are enriched for genes. We also used additional sequences of methyl-filtered reads from GenBank (mostly generated by The Maize Sequencing Consortium) in our analysis. Since the corn genome is not complete, we chose to use the sequence of the closely related rice genome as a surrogate for the corn genome in our analysis. We also compared the publicly available set of maize expressed sequence tags (ESTs) and rescue-Mu sequences available from GenBank with gene predictions from the rice genome. Figure 1 shows that after a relatively small number of sequence reads (about 60,000), methyl-filtered reads become more efficient at detecting genes than are ESTs. The data show that the methyl-filtered approach is obtaining a large percentage of the maize

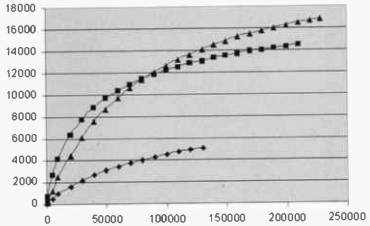

FIGURE 1 Comparative gene discovery. Random sets of maize reads (*closed squares*) MFRs; (*closed triangles*) ESTs; (*closed diamonds*) RMs. The random sets were selected and the total number of distinct rice BAC/PAC FGENESH predictions overlapped by the reads were plotted.

genome and can serve as a basis for an overall strategy to sequence large plant genomes. These results were published in 2003. We are currently refining and testing strategies to link the methyl-filtered reads to the physical map of corn and finish the genes to high quality. This strategy has the potential to produce a highly valuable, complete sequence of the corn "genespace" for a fraction of the cost of even a low-quality draft sequence of the corn genome.

Detection of Transcripts and Transcriptional Start Sites in the Mouse Genome

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Last year, we began a project to experimentally confirm the presence of mouse genes predicted by computational methods as well as to determine their transcriptional start sites. After our initial work last year, we expanded the number of genes under study and the tissues in which we assayed for their presence. A total of 300 mouse gene predictions was selected for analysis. Although some of these were well-characterized genes, a number of the predictions were not considered to be part of the canonical gene set (based on the ENSEMBL pipeline). We then placed these genes in one of five different categories based on the quality and quantity of supporting evidence for the gene model. The categories, the definitions, and the number of genes tested in each group are shown in Table 1.

TABLE 1 Description of Gene Categories

Category	Number of genes tested	Definition
EPD	13	Genes found in the Eukaryotic Promoter Database having experimentally verified transcriptional start sites.
RefSeq	27	NCBI's curated nonredundant set of genes.
Category B	23	Automated NCBI predictions covered by multiple ESTs.
Category C	169	Gene predictions covered by a single EST.
Category D	68	Gene predictions that do not overlap any EST, mRNA, cDNA, ENSEMBLE, or GENIE evidence.

Three hundred mouse genes or gene predictions were classified into five categories based on the quality of associated evidence. The definition column describes the basis for the classification. Genes in the EPD category have the highest-quality evidence and were used as positive control for all experiments. Predicted genes in Category D are considered to be based on evidence with the least amount of confidence.

Gene sequences and sequences obtained using the RACE-PCR (polymerase chain reaction) method were aligned to the mouse genome using BLAT (Kent et al., *Genome Res. 12:* 656 [2002]). A RACE-PCR sequence was counted as a positive hit if it satisfied all of the following criteria: (1) alignment indicated a spliced product, (2) mapped to the same region of the genome as the gene sequence, (3) could be mapped uniquely to the genome with >98% identity for >95% of the sequence, (4) at least two clones were obtained with similar exon structure (cases where only one clone was obtained for a particular variant were also considered a hit if the variant sequence matched an EST or cDNA deposited in GenBank), and (5) the sequence contained the RACE-specific primer sequence. Sequences for 15 genes (in C and D categories) did not produce spliced products but agreed with all of the other criteria mentioned above. Careful analysis indicated that for all of these cases, the exon that contained the primer sequences was itself the first exon. For two of these cases, spliced products were obtained using additional primers and amplifying the 3′ end of the gene using 3′ RACE-PCR. This indicates that these are real genes and therefore counted as positive hits.

The result of 5′ RACE-PCR on a set of 15 mouse tissues/stages is shown in Figure 2 (top). The genes in the EPD set serve as our internal positive control. All 13 genes in the EPD category were detected. For all of these genes, at least one splice variant was detected

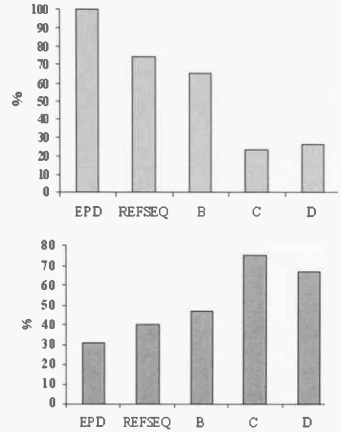

FIGURE 2 Detection of 5′ ends of genes using RACE-PCR. (*Top*) 5′ RACE-PCR was performed on 15 mouse tissues/stages. The percentage of genes detected by this method and satisfying the criteria are shown for different categories. (*Bottom*) Percentage of detected genes where the reference sequence annotation was found to be incomplete at the 5′ end is shown for each category.

that agreed with the annotated first exon for the corresponding gene in the Eukaryotic Promoter Database. A majority of the well-characterized and curated genes (100% in the EPD and 74% in the REFSEQ categories) produced spliced products.

As expected, a significantly lower percentage of class C and D genes were amplified. In the case of the RefSeq genes, of the seven genes not detected, four produced a nonspecific amplification product. This indicates a lack of primer specificity due to a related sequence elsewhere in the genome. An alternative in these cases is that the primer did not match an exon and the amplified product represents background amplification. In either case, it is likely in these cases that alternate primer selection will resolve the problem.

The simplest explanation for not detecting more of the RefSeq genes tested may be that the transcripts were absent in the tissues/stages tested or that the annotated gene structures contained sufficient errors to interfere with selecting useful primers for amplification. Initial trials using four mouse tissues (7- and 17-day embryos, total brain and testes) resulted in the detection of only 91 genes. The detection rate improved by 5% when the number of tissues tested was increased to 15, indicating that tissue distribution is clearly an issue. For class C and D genes, many of the failures probably were due to attempts to amplify something that really is not a gene. Since the class C and D genes are not considered to be "real" in that they are not in the canonical gene set, their detection even at this level is somewhat astonishing, suggesting that there are a significant number of genes not currently annotated. Moreover, Figure 2 (bottom) shows the percentage of gene annotations which agree with the experiemental data. It shows that even in the case of RefSeq genes, a substantial number of genes have transcriptional start sites that differ from those shown in the current database. In some cases, these likely represent alternative 5′ exons, perhaps expressed in a tissue-specific manner. In a number of cases, however, the discrepancies likely represent errors in the databases. These discrepancies are highly problematic for future bioinformatics analyses of the mammalian transcriptome. Ideally, one would like to correlate the sequences of the promoters of genes with their expression based on microarrays or other data. This requires an accurate determination of the transcriptional start site of the gene, and our data indicate that this may frequently be lacking in the current databases.

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ADVANCED COMPUTING TOOLS FOR BIOLOGY

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Modern biological research has primarily focused on our capability for generating massive amounts of *syntactic* information from genomics and functional genomics data, but it has been slow in exploiting this capability to unravel their *semantics*. This deciphering requires a powerful information technology infrastructure for translating our understanding of the *part lists* to a deeper understanding of their interactions at a systems level.

Tools that are capable of uncovering subtle and universal biological principles at play will enable revolutionary progress in the biomedical sciences. Creating these tools has become one of the grand challenges in computer science today.

This group has focused its efforts on building tools across the gamut of modern computational biology. Our core tool for biological discovery is a multi-scripting environment called Valis. Valis allows bioinformatics researchers to collaborate by providing core bioinformatics tools and access to numerous statistical, numerical, and visualization libraries through simple scripting languages. In fact, a single program can call routines written by other researchers in other languages with no extra coding necessary. Valis now incorporates the Simpathica "systems biology" tool-set created for the study of biological pathways and evolutionary processes. The group also has created NYUMAD (Micro Array Database) a tool for collecting, analyzing, and distributing microarray data.

The introduction of information technology into biology and biotechnology has already transformed and accelerated the nature of biological research. The impact of this transformation has been felt in the areas of agriculture, pharmacology, disease diagnosis and prognosis, forensics, defense against bio-warfare, and biometry, and ultimately affects how we interact with our immediate natural environment. Drawing on ideas from systems sciences, dynamical systems, logic, computer science, and many other physical sciences, there is now an opportunity to build tools that are mathematically precise, sound, and exhaustive, computationally efficient, and scalable.

VALIS

One of the central efforts of the group has been to create a bioinformatics environment that will make it easier for biologists to develop their own computational tools. This environment, called Valis, includes tools for visualization of biological information, design and simulation of in silico experiments, and storage and communication of biological information. Listed below are novel features in the Valis environment.

- **Language-independent Architecture:** The Valis advanced scripting engine can integrate research from multiple groups into a single environment. Researchers using the Valis framework can share both the data and the algorithms for the analysis of that data. The language-independent architecture of Valis allows research groups to leverage programs written in different languages.
- **Whole-genome Analysis:** New data structures and algorithms make it possible to perform whole-genome analysis on commodity hardware. As the throughput of sequencing efforts increases, Valis is likely to become the only tool available that allows analyses to be performed on entire genomes.

Valis, which is slowly being rolled out to the larger scientific community, has recently been used to develop a set of methods that can validate and find discrepancies in recently released human genome sequence data.

These methods will have an immediate and direct impact on the biological community: creating algo-

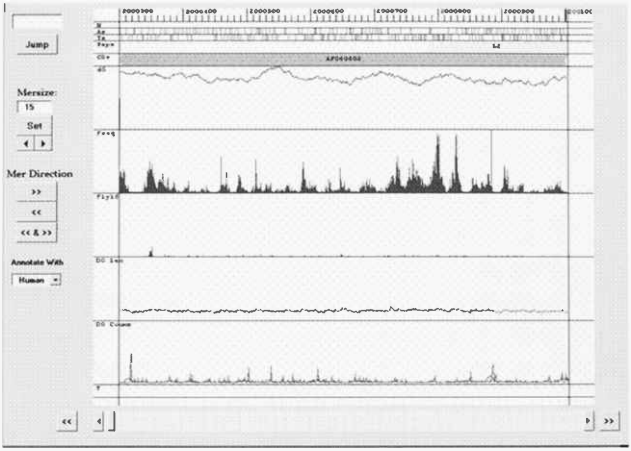

FIGURE 1 Image of a simple genome browser created in the Valis environment. Bands above show genes, mer-frequency analysis, and Gibbs free-energy along a segment of the genome. Using modern computer science techniques and seamless integration with cluster-computers, the genome browser allows whole-genome analyses to be performed on the fly.

rithms for understanding and extracting information from genomic and transcriptomic data in a coordinated manner; building, modifying, and correcting existing models to understand biological processes; and creating a common and unified language for biologists to communicate, exchange data, design, and disseminate experimental protocols.

SIMPATHICA

The Simpathica system allows biologists to construct and simulate models of metabolic and regulatory networks and analyze their behavior. Metabolic pathways can be drawn on the screen through a visual programming environment or, in a specialized XML format, a language originally designed to promote information exchange between multiple systems and programs. The system allows a biologist to combine simple building blocks representing well-known objects: biochemical reactions and modulations of their effects. The system then simulates the pathways thus entered. Coupled with a natural language system, the Simpathica tool allows a user to ask questions, in plain English, about the temporal evolution of the pathways previously entered.

Collaborators at Cold Spring Harbor Laboratory have recently used Simpathica to explore a *Caspase cascade* model for apoptosis, cell death. Using the system, a rough topological model was constructed and analyzed. This simple model pointed out the possible presence of several unmodeled processes, without which the core model could not explain various well-known threshold effects.

Using modeling tools like Simpathica to simulate biological processes in silico, a biologist can model and study the behavior of complex systems, exploring many different scenarios rapidly without relying solely on experimentation. Tools like Simpathica will allow researchers to formulate a hypothesis and design better experiments, drastically cutting down the time for research. Simpathica now includes a natural language interface to allow the system to be queried in plain language.

lular communication processes (e.g., cell signaling involved in cancer and development).

- Analyzing genomic information from microbes to humans and the evolutionary forces that shaped them.
- Using genomic, proteomic, and biochemical information to aid in the process of understanding disease processes.

This interdisciplinary group places an equal emphasis on computational techniques and biology and therefore attracts researchers and students from mathematics, statistics, computer science, and biology who collaborate with physicians, physicists, and chemists from leading national laboratories in bioinformatics and in industry. Through the consilience of many disciplines, this group hopes to advance the entire biological community through its software (Valis, NYUMAD, Simpathica, etc.).

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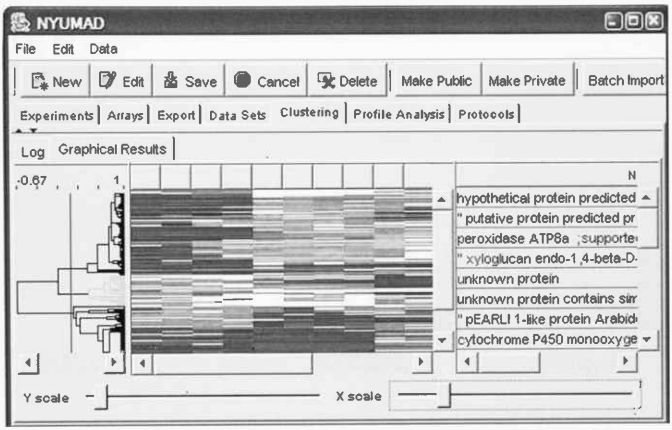

FIGURE 2 Image of NYU MicroArray Database Application.

NYU MICROARRAY DATABASE

Our group has also developed a sophisticated software system to maintain and analyze biological abundance data (e.g., microarray expression levels or proteomic data) along with associated experimental conditions and protocols. The prototypic system is called the NYU MicroArray Database (NYUMAD) and is being expanded to deal with many other related experiments. It uses a relational database management system for the storage of the data and has a flexible database schema designed to store any type of abundance data, along with general research data such as experimental conditions and protocols.

The database schema is defined using standard SQL (Structured Query Language) and is therefore portable to any SQL database platform. NYUMAD supports the MAGE-ML standard for the exchange of gene expression data, defined by the Microarray Gene Expression Data Group (MGED) and is accessible via the Web.

NYUMAD is a secure repository for both public and private data. Users can control the visibility of their data. Initially, the data might be private, but after publication of the results, the data can be made visible to the larger research community. Data analysis tools are supplemented with visualization tools. The goal is not only to provide a set of existing techniques, but to incorporate ever more sophisticated and mathematically robust methods in the data analysis and to provide links and integration with our other tools such as the Valis system.

Our goal for NYUMAD is to create a set of centralized repositories allowing researchers to search for patterns in gene behavior over large samples collected at many different sites. We hope that NYUMAD will become a comprehensive resource for biological abundance data that is as easily queried as the World Wide Web.

SUMMARY

In the "postgenomic" frontier of biological research, computer science has a critical role in advancing biological discovery, and the group intends to stay on the cutting-edge of this research. We aim to create better computational biology tools by focusing on more accurate and efficient mathematical methods for analyzing genetic data.

A summary of the biological problems the group tackles include the following:

- Understanding regulatory, metabolic and intercel-

EVOLUTIONARY CONSTRAINTS AS PROTEIN PROPERTIES REFLECTING UNDERLYING MOLECULAR MECHANISMS

A.F. Neuwald N. Kannan

Our understanding of protein function comes through analyses of their basic properties, such as their sequences, structures, and subcellular locations. Rather than examining physical properties, however, our focus is on quantifying and characterizing a protein's evolutionary properties, namely, the sequence constraints imposed by natural selection during evolution. Such constraints are evident as sequence patterns conserved in representative members of a protein family that have diverged from a common ancestor at least a half billion years ago. Such proteins would have lost all sequence similarity if not for the selective pressures maintaining underlying mechanisms important for their biological functions.

Because we focus on classes of proteins for which vast amounts of sequence, structural, and other experimental data are available, powerful statistical procedures can be used to detect subtle yet significant characteristics. These are applied along with other procedures to (1) detect and align distantly related sequences, (2) categorize the conserved patterns present in those sequences, (3) quantify the associated selective constraints, and (4) interpret these constraints in light of available structural, biochemical, taxonomic, and genetic data. We term this approach contrast hierarchical alignment and interaction network (CHAIN) analysis, an initial description of which was published early this year. In this publication, we also illustrate the application of CHAIN analysis to Ras-like GTPases, a family of proteins important to our understanding of cancer.

A MATHEMATICALLY RIGOROUS DESCRIPTION OF EVOLUTIONARY CONSTRAINTS

This work was done in collaboration with Jun Liu (Department of Statistics, Harvard University). Although even a qualitative description of selective constraints is informative, a mathematically rigorous description can reveal subtle aspects of protein function that might otherwise be missed. For this reason, we developed a statistical description of protein constraints analogous to that formulated for Gibbs-free energy in statistical thermodynamics. Just as the energy of a chemical reaction is defined by the degree to which reactants are shifted toward products, we have mathematically defined selective constraints by the degree to which the proteins of interest have been shifted away from related proteins during evolutionary divergence. We similarly define differences in selective constraints between related subgroups of proteins in a manner roughly analogous to free-energy changes between thermodynamic states. Important aspects of our statistical description are algorithmic inasmuch as valid quantification of selective constraints strongly depends, for example, on the accuracy of the multiple sequence alignments used to measure them. In a submitted manuscript, we describe these statistical and associated algorithmic procedures, including a hidden Markov model, an algebraic system, and Monte Carlo sampling strategies for gapped multiple alignment of protein sequence motifs. This work corrects certain misaligned regions (associated with proteins harboring sizable insertions) that were encountered when using the PSI-BLAST multiple alignment method originally implemented for CHAIN analysis (see Fig. 1). This paper also describes intervention strategies that improve a multiple alignment based on expert knowledge provided by the user. Several programs implementing these procedures are being made available, including GISMO (*Gi*bbs-like *s*ampling with *m*ultiple *o*perations) and GARMA (genetic *a*lgorithm for *r*ecombinant *m*ultiple *a*lignment). Another significant algorithmic breakthrough implemented in the GARMA program is a statistical procedure that automatically optimizes gap penalties.

CHAIN ANALYSIS OF DNA POLYMERASE III CLAMP LOADING

The prokaryotic DNA polymerase III clamp loader complex loads onto DNA and unloads the β-clamp, which links the replication complex to DNA during polynucleotide synthesis. The minimal complex necessary for clamp loading consists of one δ, one δ′, and three γ subunits, which are evolutionarily related,

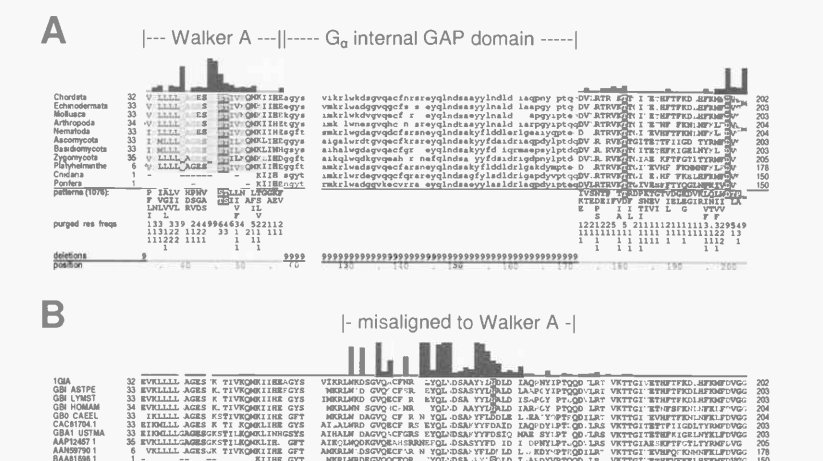

FIGURE 1 Correction of a protein multiple alignment error within a G-GTPase harboring an inserted GTPase activation protein (GAP) domain. (*A*) Corrected CHAIN analysis alignment using our new procedures. Both the Walker A and the Walker B (unlabeled) motifs are correctly aligned. (*B*) Uncorrected alignment generated using PSI-BLAST. Note that the Walker A motif is misaligned against the carboxy-terminal region of the GAP domain.

although only the γ subunit exhibits ATPase activity. We have applied CHAIN analysis to the β-clamp and to the γ and δ′ clamp loader subunits. A paper describing the β-clamp analysis has been published. This analysis reveals conserved residues that appear to be associated with various aspects of β-clamp loading onto and unloading from DNA. This, in turn, suggests corresponding mechanisms, including, for example, how binding to DNA may be coupled to closing of the clamp. Although CHAIN analysis is at present typically applicable only to very large classes of proteins, the β-clamp paper illustrates that it nevertheless sometimes works for a relatively small set of proteins (~50 distinct β clamps). This was achieved by structurally aligning three homologous domains present in β-clamp monomers (which thus increased the effective number of β domain sequences aligned to 150).

This supports our conviction that CHAIN analysis will be more widely applied as we better understand the principles involved in identifying and interpreting evolutionary constraints. CHAIN analysis of the DNA polymerase III γ and δ′ subunits similarly reveals selective constraints acting upon the clamp loader complex. Categorization of these constraints points to specific residues likely involved in contacts between subunits of the γ complex, in coordinated conformational changes associated with clamp loading, and in coupling binding to DNA with ATP hydrolysis.

CMGC PROTEIN KINASE STRUCTURAL MECHANISMS

Eukaryotic protein kinases propagate and amplify extracellular and intracellular signals and thus have

important regulatory roles in diverse cellular processes, such as metabolism, transcription, cell cycle progression, apoptosis, and neuronal development. We have continued to apply CHAIN analysis to various classes of protein kinases, including the MAP, CDK, GSK, and SR protein kinase families. This has revealed both important family-specific mechanistic features, including a possible role for conserved buried waters in substrate specificity, and group-specific mechanisms, including a network of conserved interactions that appears to couple CMGC kinase activation to regulatory factors that bind via a characteristic insert region. We were invited to present this analysis at the 2003 Protein Phosphorylation Workshop in Asilomar, California. The organizers of this meeting have asked us to incorporate the results of this and similar analyses into the Protein Kinase Resource (http://pkr.sdsc.edu/html/index.shtml).

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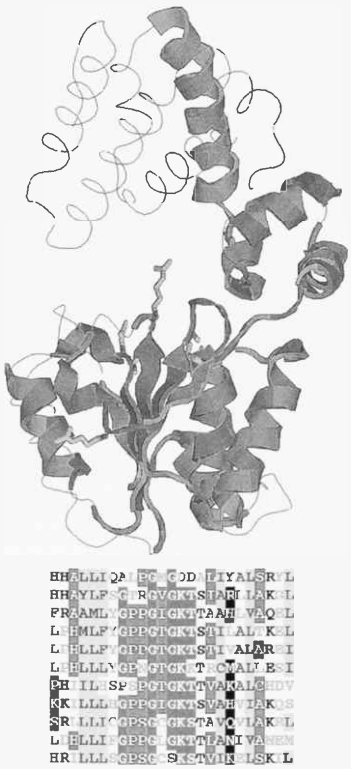

Structure of an inactive ATPase proportional to the degree of sequence conservation among homologous proteins.

COMMUNITY ACCESS TO GENOME RESOURCES

L. Stein

GRAMENE: A COMPARATIVE MAPPING RESOURCE FOR GRAINS

The Gramene database (www.gramene.org) is a comparative mapping resource for rice and other grains. Gramene allows researchers to compare the genetic and physical maps of the major monocot crops, namely, maize, barley, oats, sorghum, and wheat, to the emerging rice genomic sequence. This allows researchers to identify candidate genes in the rice genome that correspond to genetically mapped mutants and quantitative traits in the non-rice crop they are studying. Hence, the resource grants researchers studying traits in maize, barley, and so forth the benefit of genomic sequencing without waiting for the sequencing of these much larger genomes.

In addition to comparative maps, Gramene offers up-to-date genomic annotation of the rice genome, including both predicted and confirmed genes, and the current physical maps of rice and sorghum. We have mapped more than 1 million monocot ESTs (expressed sequence tags) to the rice genome, allowing gene predictions to be further refined based on cross-species comparisons. During this year, we developed a new integrated map of the rice and maize genomes that allows researchers to move back and forth between various genetic and physical maps of these species in order to apply the knowledge developed in one organism to finding functionally significant genes in the other.

Curation is an important component of Gramene. In collaboration with Susan McCouch's laboratory in Cornell, we have curated more than 12,000 rice proteins, assigning them classifications in the Gene Ontology (www.geneontology.org). In addition, we have classified more than 700 rice mutants using a trait ontology that we have developed. We are currently curating the rice biological literature, much of which is in non-English languages, and making this information available via Gramene as well.

During the past year, Gramene increased its holding substantially by adding a variety of important comparative mapping resources, including high-throughput gene-rich sequences from maize and sorghum, and a series of mapped transposon insertions useful for functional genomics. Our work culminated this year in coauthorship in a *Science* paper on the sequencing of Rice Chromosome 10.

WORMBASE: A RESOURCE FOR *C. ELEGANS* GENOME AND BIOLOGY

Our lab continues to be a major developer and maintainer of the WormBase database, an on-line information resource for the small free-living nematode, *Caenorhabditis elegans*. This organism is favored as a simple model animal because of its small genome size, experimental malleability, and well-understood cellular anatomy. WormBase is a curated model organism database developed as part of an international collaboration that includes the California Institute of Technology, Washington University at St. Louis, and the Sanger Centre. Our lab is responsible for the Web site, user interface, and software architecture for the project.

The resource, which is available to the public at www.wormbase.org, contains the complete *C. elegans* genome and key annotations, including predicted genes, alternative splicing patterns, oligonucleotide probes, and evolutionarily conserved segments. It also contains many other types of biological information, including the *C. elegans* cell pedigree, the organism's neuroanatomy, its genetic map, and the physical map from which the genomic sequence was derived.

Our major work during 2003 was to use WormBase as a platform to analyze the genome sequence of *C. briggsae*, a sister species of *C. elegans* that diverged approximately 100 million years ago. Our work on *C. briggsae* has resulted in new insights into the evolution of genomes and gene families, particularly with respect to the functionally important chemsensory receptor family of genes (Fig. 1). The analysis also allowed us to add approximately 1000 new genes to *C. elegans* and to correct and improve several thousand others. This work resulted in a major publication in the new journal *PLoS Biology*.

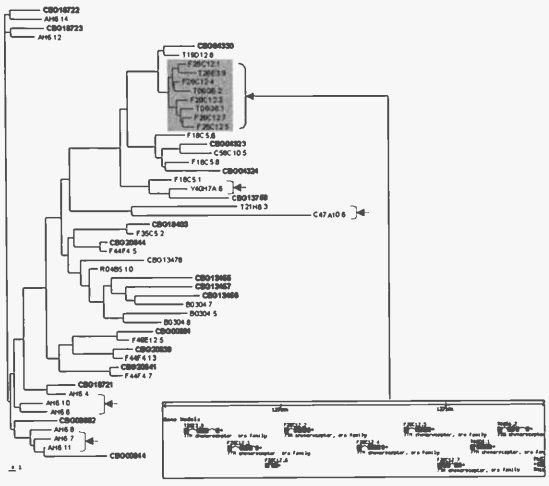

FIGURE 1 Tree view of all chemosensory receptor genes in the sra subfamily. *C. elegans* is shown in white background and *C. briggsae* in light gray background. The arrows indicate regions of *C. elegans*-specific expansion of the family. (*Inset*) Schematic of the region of *C. elegans* chromosome I corresponding to the sra expansion in the upper right of the tree. *C. briggsae* genes are named using the prefix CBG, whereas *C. elegans* genes are numbered consecutively across the cosmid on which they were first identified. This analysis shows evolution in action: The chemosensory receptor gene family is actively growing in *C. elegans* by a process of tandem duplication.

GENOME KNOWLEDGEBASE

The Genome KnowledgeBase (GKB) is a collaboration with the European Bioinformatics Institute (EBI) and the Gene Ontology Consortium to develop a Web-accessible resource for curated information about biological processes.

The GKB is organized like a review journal. Experimental biologists are invited to create "summations" that summarize a particular aspect of their field. Currently, summations include "DNA Replication," "Protein Translation," and the basic pathways of intermediate metabolism, such as the TCA cycle. New modules for "RNA Splicing" and "Cycle Checkpoints" are in progress. Summations are similar to mini-reviews, except that each paragraph of text is reduced to a series of logical assertions that is entered into a database of processes and macromolecules. The database is then used to drive a Web site. The Web site can be browsed like a textbook or searched with queries to discover pathways and connections.

During 2003, we roughly doubled the number of modules described in GKB, thereby covering about 80% of intermediate metabolism and 75% of the Central Paradigm. We held a total of five "annotation jamborees" in New York and London, meetings at which researchers and GKB curators worked side-by-side to create new modules and troubleshoot existing ones. In parallel with this work, we significantly improved the visualization tools available on the GKB Web site. In particular, the "Constellation Viewer" (Fig. 2) provides a birds-eye view of all macromolecular reactions known to the database, simultaneously providing a navigation tool for researchers and an advanced visualization tool for hypothesis generation. In the near future, it will be possible to superimpose large-scale data sets, such as microarray experiments, onto the Constellation Viewer in order to see at a glance which pathways are affected.

GENERIC MODEL ORGANISM DATABASE PROJECT

In collaboration with the model organism system databases FlyBase, SGD, and MGD, the Generic Model Organism Database (GMOD) project is developing a set of database schemas, applications, and interfaces suitable for creating a model organism system database. The hope is to significantly reduce the time and expense required to create new databases to curate genomic information coming out of various model organism system sequencing projects (e.g., rat, *Dictyostelium*, and *Plasmodium*).

The end of calendar 2003 saw the first alpha release of the GMOD package, consisting of a modular database, Chado, a genome browser, GBrowse, and a genome editor, Apollo. The package includes documentation, installer scripts, and example data and is currently being tested and scrutinized by a number of academic and commercial groups. Partially in recognition of the value of the GMOD project, Stein was awarded the Benjamin Franklin Award in Bioinformatics, given annually by Bioinformatics.org, for his promotion of open source and open standards tools.

THE HUMAN HAPLOTYPE MAP

The International Human HapMap Project (http://www.hapmap.org) seeks to map out regions of common genetic variability in the human genome by genotyping three major world populations at a resolution of 1 marker per 5 kbp. When complete, the resulting "haplotype map" will greatly reduce the cost of genetic association studies to find cancer susceptibility genes and other disorders with genetic components.

FIGURE 2 Visualizing pathways with the Genome KnowledgeBase. Shown is a page from the Cell Cycle Checkpoints module. At the top is the Constellation View. All pathways known to the Knowledgebase are displayed in gray, and those relevant to cell cycle checkpoints are highlighted. As the user magnifies the Constellation View, more detail appears. At the bottom is the main page, where the user is shown detailed information on the first steps of the pathway.

Our lab is a central participant in this project in our role as the Data Coordinating Center (DCC). We manage the central database for the project, allocate SNPs to the 11 genotyping centers, coordinate data submission, quality checks, and quality control, and mange the public release of project data. The HapMap Web site, which was developed in our lab, describes the project in the four languages of the project participants (English, French, Chinese, Japanese, Yoruba) and provides access to the data both for bulk download and for interactive querying and browsing using the GMOD tools.

A policy paper describing the project, its goals, and the data management issues was published in a December 2003 article in *Science*.

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COMPUTATIONAL GENOMICS

M.Q. Zhang N. Banerjee, Y. Benita, G.X. Chen, D. Das, R. Das, N. Hata, H. Lin, K. Mamoru, D. Schones, F. Wang, J.H. Wang, Z.Y. Xuan

Our research interest continues to be the identification and characterization of the genetic elements in nucleic acid sequences by computational means. This last year, our main focus was to continue building our own large-scale genome analysis infrastructure. After the first-pass annotation of the human promoters, we have been further developing our promoter annotation pipeline to include comparative genomics; it can combine FirstEF prediction, mRNA/EST (expressed sequence tag) data, and other mammalian genomic sequences to annotate promoters and first exons for human, mouse, and rat simultaneously and automatically. We built the first version of CSHL Mammalian Promoter Database and a promoter data set for Affymetrix gene chip HGU133A. We have also been doing genome-wide comparative analyses of alternatively spliced transcripts between human and mouse and constructing a Mammalian Alternatively Spliced Transcript Database. In collaboration with the Krainer lab here at CSHL, we have built *ESEfinder*, a Web resource, to identify exonic splicing enhancers. We have developed new computational methods for transcription factor binding-site motifs by taking all three large-scale resources: comparative genomic sequences, ChIP-chip and expression microarray data. First of all, we developed a novel motif-finding algorithm called *BEAST*, which allows users to identify differentially "expressed" motifs between two arbitrary (target and control) sequence sets. This tool has been successfully applied to find cooperative motifs when combining sequence, expression, and ChIP-chip data or to find functionally more relevant motifs when combining expression and GO annotation data. We have also demonstrated that cooperative transcription factors may also be identified directly without using sequence data. In collaboration with the Ren lab at the University of California, San Diego (UCSD), we have found a global transcriptional regulatory role for c-Myc in Burkitt's lymphoma cells. In collaboration with the Fu lab at UCSD and the Fan lab at Illumina, we have collected the data from our first alternative splicing chip experiments to compare transcript isoform differences between prostate cancer and normal cells; analysis is still being carried out at the moment. Below are more detailed descriptions of some finished projects in 2003.

We (Z.Y. Xuan and M.Q. Zhang) are also collaborating with the McCombie and Hannon labs here at CSHL to systematically examine gene predictions in the mouse genome.

Annotation of Promoters and First Exons in the Human Genome

I. Grosse, M.Q. Zhang [in collaboration with R. Davuluri, Ohio State University]

The human genome contains a vast number of *cis*-regulatory regions responsible for directing spatial and temporal patterns of gene expression, and delineating their locations is of paramount importance for our understanding of gene expression and regulation. However, due to the absence of full-length 5′-untranslated regions for many of the cDNAs, the identification and annotation of these functional regions are behind that of coding regions. Here, we present an initial computational annotation of promoters and first exons for the 24 chromosomes of the human genome. Using expressed sequence databases and an automated annotation pipeline, we have annotated promoter and first exons for a total of 53,645 different transcripts in 34,255 genes. In current annotations, 80% of the identified genes are supported by RefSeq/mRNA or spliced EST transcripts; and 30% of the genes have two or more alternative first exons and associated splice variants.

Identification of Cooperativity Among Transcription Factors Controlling Cell Cycle in Yeast

N. Banerjee, M.Q. Zhang

Transcription regulation in eukaryotes is known to occur through the coordinated action of multiple tran-

scription factors (TFs). Recently, a few genome-wide transcription studies have begun to explore the combinatorial nature of TF interactions. We propose a novel approach that reveals how multiple TFs cooperate to regulate transcription in the yeast cell cycle. Our method integrates genome-wide gene expression data and chromatin immunoprecipitation (ChIP-chip) data to discover more biologically relevant synergistic interactions between different TFs and their target genes than previous studies. Given any pair of TFs *A* and *B*, we define a novel measure of cooperativity between the two TFs based on the expression patterns of sets of target genes of only *A*, only *B*, and both *A* and *B*. If the cooperativity measure is significant, then there is reason to postulate that the presence of both TFs is needed to influence gene expression. Our results indicate that many cooperative TFs that were previously characterized experimentally indeed have high values of cooperativity measures in our analysis. In addition, we propose several novel, experimentally testable predictions of cooperative TFs that have a role in the cell cycle and other biological processes. Many of them hold interesting clues for cross-talk between the cell cycle and other processes, including metabolism, stress response, and pseudohyphal differentiation. Finally, we have created a Web tool where researchers can explore the exhaustive list of cooperative TFs and survey the graphical representation of the target genes' expression profiles. The interface includes a tool to dynamically draw a TF cooperativity network of 113 TFs with user-defined significance levels. This study is an example of how systematic combination of diverse data types, along with new functional genomic approaches, can provide a rigorous platform to map TF interactions more efficiently.

A Global Transcriptional Regulatory Role for c-Myc in Burkitt's Lymphoma Cells

M.Q. Zhang [in collaboration with the Ren lab, University of California, San Diego]

Overexpression of c-Myc is one of the most common alterations in human cancers, yet it is not clear how this transcription factor acts to promote malignant transformation. To understand the molecular targets of c-Myc function, we have used an unbiased genome-wide location-analysis approach to examine the genomic binding sites of c-Myc in Burkitt's lymphoma cells. We find that c-Myc together with its heterodimeric partner, Max, occupy >15% of gene promoters tested in these cancer cells. The DNA binding of c-Myc and Max correlates extensively with gene expression throughout the genome, a hallmark attribute of general transcription factors. The c-Myc–Max heterodimer complexes also colocalize with transcription factor IID in these cells, further supporting a general role for overexpressed c-Myc in global gene regulation. In addition, transcription of a majority of c-Myc target genes exhibits changes correlated with levels of c-*myc* mRNA in a diverse set of tissues and cell lines, supporting the conclusion that c-Myc regulates them. Taken together, these results suggest a general role for overexpressed c-Myc in global transcriptional regulation in some cancer cells and point toward molecular mechanisms for c-Myc function in malignant transformation.

ESEfinder: A Web Resource to Identify Exonic Splicing Enhancers

J.H. Wang, Z.W. Zhu, M.Q. Zhang [in collaboration with the Krainer lab, Cold Spring Harbor Laboratory]

Point mutations frequently cause genetic diseases by disrupting the correct pattern of pre-mRNA splicing. The effect of a point mutation within a coding sequence is traditionally attributed to the deduced change in the corresponding amino acid. However, some point mutations can have much more severe effects on the structure of the encoded protein, for example, when they inactivate an exonic splicing enhancer (ESE), thereby resulting in exon skipping. ESEs also appear to be especially important in exons that normally undergo alternative splicing. Different classes of ESE consensus motifs have been described, but they are not always easily identified. ESEfinder (http://exon.cshl.edu/ESE/) is a Web-based resource that facilitates rapid analysis of exon sequences to identify putative ESEs responsive to the human SR proteins SF2/ASF, SC35, SRp40, and SRp55 and to predict whether exonic mutations disrupt such elements.

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The human brain is able to solve certain computations that remain far beyond today's most sophisticated computers. Anthony Zador's lab is probing the brain's unique computational style by examining how the brain processes complicated auditory input we call sound. Zador's lab has uncovered a code that neurons use to represent sound and is working to sort out the different excitatory and inhibitory components that generate the code.

Dmitri Chklovskii's lab uses mathematics and physics to examine constraints on brain structure and function. By uncovering such limits, they hope to delineate the "engineering" principles that govern brain evolution. The scientists ask how neurons are connected and are working to understand how neurons organize themselves into circuits and how those circuits produce behavior. By comparing the patterns of brain structure between animals, they have begun to pinpoint features that are fixed and characteristics that can vary. This year, they made great progress in determining the rules that specify several different brain characteristics, including those that govern where neurons are placed and how they are connected to each other.

Partha Mitra and his colleagues are pioneering the field of neuroinformatics, which involves the development of software and mathematical algorithms for interpreting large amounts of data from electrophysiological, brain imaging, and animal behavior studies. Another of Mitra's interests is "theoretical engineering" in which he attempts to comprehend specific biological phenomena—such as the comparitively high metabolic rates found in neural tissue—by using mathematical tools from traditional engineering disciplines.

By focusing on a part of the brain that is variable or "plastic," Hollis Cline's lab examines how sensory experience shapes the ways neurons connect during development. Cline and her colleagues study the development of the visual system in amphibian tadpoles. They address how sensory activity stimulates mechanisms within brain neurons and thereby guides visual system development. This year, they found that normal development of links between the eye and the central nervous system requires the activity of one specific receptor after visual stimulation. Many animals share mechanisms of brain development. Therefore, Cline's research is revealing fundamental processes that govern brain development in a wide range of species, including humans.

Karel Svoboda's lab investigates how sensory experience triggers the long-term changes in brain architecture believed to underlie memory. Using custom-made state-of-the-art imaging technology, the scientists have taken pictures of synaptic connections between neurons in the brains of adult mice. Svoboda and his colleagues have found that as animals age, synapses in a particular brain region lose their ability to change shape and rewire themselves in response to input, providing specific, direct evidence for the basis of age-related memory loss.

Roberto Malinow's lab studies how the molecular composition of synapses changes in response to sensory experience. Perhaps their most exciting recent finding was their demonstration, using intact animals, that experience drives neurotransmitter receptor proteins called AMPA receptors into synapses. This is the first experience-induced specific molecular modification of a synapse to be discovered. The Malinow lab is now elucidating the roles of proteins called PSD-95 and NR2B in AMPA receptor trafficking or movement into synapases. Malinow's findings indicate that dynamics of AMPA receptor replacement at synapses is likely to be a principal mechanism of learning and memory.

Josh Huang's lab has focused on a specific type of inhibitory synapse or "off switch" defined by a molecule that it releases (GABA). They have developed techniques for studying these special synapses in animals and in cell cultures, which allow them to investigate how a myriad of crucial "off" switches are built into complex brain circuitry during development. They have made great progress in revealing the relative contributions of a "hard-wired" genetic program versus experience-dependent sensory inputs to the placement, molecular composition, and connectivity of neurons that respond to GABA as the brain develops.

Because the same proteins implicated in human Alzheimer's disease are present in the fruit fly brain, Yi Zhong is using the power of fruit fly genetics to unravel the causes of Alzheimer's disease in humans. Zhong's group has found that buildup of one particular protein (Aβ42) is sufficient to cause nerve cell death and memory loss in flies. Now they are trying to find genes that can prevent or reverse the damage. Zhong's "Alzheimer's Flies" can be used to identify new molecular targets for treating the disease or to screen large numbers of drugs for those that might slow or prevent nerve cell death in the brain of Alzheimer's patients.

Tim Tully's lab studies learning and memory by using genetics to isolate fruit fly mutants ("Pavlov's Flies") that display defects in memory formation. They have also used DNA microarrays to uncover genes that might be involved in memory. In this way, Tully, Josh Dubnau, and their colleagues have identified dozens of genes that function to form memories in flies. Many of the memory genes have counterparts in mice and humans, confirming that Tully has tapped into the genetic basis of a fundamental memory mechanism.

Dubnau also uses fruit flies as a powerful "model system" for investigating the fundamental mechanisms that govern learning and memory. By using a variety of approaches, his lab is investigating several genes and corresponding biochemical pathways that affect memory. Moreover, to identify and better understand the complicated individual neural circuits involved in the different stages of memory, they are using a technique that enables them to switch specific groups of neurons in the fly brain on and off at will.

Jerry Yin's lab investigates a process believed to underlie memory called synaptic tagging. Yin's lab is interested in how the interaction of particular proteins during synaptic tagging drives the formation and maintenance of memory. They have characterized several proteins that might control memory formation and have investigated how neurons might confine proteins to particular synapses via synaptic tagging.

Alexei Koulakov is working to mathematically model components of brain circuitry that act not in sensing (sensory neurons) or in acting on information (motor neurons), but rather in deciding what to do (decision-making neurons). His lab has successfully modeled some of the key properties that such decision-making neurons are predicted to have, and Koulakov has designed a real-world experimental strategy for studying such neurons in vivo.

Carlos Brody uses a combination of mathematics, animal experiments, and human psychophysical approaches to explore short-term memory including how short-term memory is linked to decision-making processes, and how the brain senses and represents time. Brody and his colleagues have developed a number of mathematical models that have enabled them to reveal the probable neurological basis of a variety of real-world perceptual and cognitive processes.

Zachary Mainen's lab studies rodent behavior that is influenced by smell to explore awareness, motivation, and decision-making. The group studies small and large-scale events, from how single olfactory neurons send out signals, to how large ensembles of neurons enable rats to make decisions based on what they smell. Ultimately, Mainen will apply the insights learned from this research to psychiatric diseases in humans, especially schizophrenia.

Taste buds are so familiar to us that they are easily taken for granted. Nevertheless, they are complex sensory organs with a unique pattern of development about which little is known at the molecular level. CSHL Fellow Lee Henry investigates the molecular mechanisms that control taste receptor cell development. By using DNA microarray technology to profile gene expression in taste bud cells, Henry has begun to decipher new principles of sensory cell development and function.

By studying adult stem cells, or unspecialized cells that can develop into many different cell types, Grigori Enikolopov's lab has found that the compound nitric oxide can instruct stem cells to stop dividing and become neurons and that decreasing the amount of nitric oxide in the brain can increase the number of stem cells and neurons. Enikolopov's studies support the notion that coaxing stem cells down particular developmental pathways might be an effective strategy for treating brain disease or injury.

COMPUTATIONAL SYSTEMS

C.D. Brody S. Chakraborty C. Machens
C. Hollweg A. Penel

Our lab is interested in how neurons interact with each other to form networks that underlie behaviors such as decision-making and short-term memory. We are also interested in how time and temporal patterns are sensed and represented in the brain. During 2003, Carlos Brody (PI) and Christian Machens (postdoc) developed a theoretical framework for understanding how neuronal responses can be rapidly and easily modulated from short-term memory responses to decision-making responses during a sequential discrimination task. Santanu Chakraborty (Watson School graduate student), continued his collaboration with David Tank (Princeton), studying network properties of neurons in the goldfish brain that support the short-term memory of eye positions. Amandine Penel (postdoc), working with Christopher Hollweg (intern), continued her investigation of how temporal patterns, defined as a train of brief sound pulses, are perceived by humans. Finally, in separate projects, Machens collaborated with the Zador lab here at CSHL to complete a paper, now in press, on the use of natural sounds to discover the structure of receptive fields of cortical auditory neurons. Brody, collaborating with John Hopfield (Princeton), completed a paper, now in press, on the learning of spike-timing-dependent plasticity rules.

A Neural Model of Sequential Discrimination

C. Machens, C.D. Brody

Sequential discrimination tasks are widely used in psychophysical studies. In a typical such task, a subject is presented with a first stimulus (f1) and then after a delay of a few seconds, with a second stimulus (f2), after which the subject must make a decision based on a comparison of the two (f2 > f1?). Although simple, this task requires at least three different components: (1) loading a stimulus with a particular value (f1) into a short-term memory system; (2) maintaining that memory value over a few seconds; and (3) when stimulus f2 is presented, computing a comparison between f2 and the memory of f1. Neurons that participate in all three components of a somatosensory sequential discrimination task have been found in the prefrontal cortex of macaques (C.D. Brody, R. Romo et al., unpubl.). On the basis of two mutually inhibiting classes of neurons, we have constructed a simple network model of prefrontal cortical neurons designed to maintain a short-term memory of f1. Surprisingly, we find that the model, built to account for the memory component of the task, also accounts for the loading and stimulus comparison components of the task. This simple model thus agrees with key aspects of the electrophysiological evidence and is able to carry out all three components of the task (loading, storing, and making a decision based on a comparison) within a single, integrated framework. The model also suggests how the experimentally observed memory-dependent and time-dependent activities may coexist with each other without disturbing the content of the memory and makes a series of experimental predictions.

Correlations between Persistent Activity Neurons in the Oculomotor System of the Goldfish

S. Chakraborty [in collaboration with D. Tank, Princeton University]

Persistent activity, defined as stimulus-dependent neural activity that persists after the end of a transient stimulus that initiates it, is thought to be the neural basis of short-term memory—memories that last on the order of several seconds. Such activity has been found in many brain areas in primates and rodents and is also found in area I of the brain stem of goldfish. In this area, persistent activity is thought to encode the memory of eye position during fixations and between saccades (rapid eye movements), which in the goldfish occur every few seconds. Neurons in area I have firing rates that, above a certain minimum threshold that varies with each neuron, are proportional to eye position. Some neurons have firing rates that increase as the eye fixation position is increasingly to the right, whereas other neurons have firing rates that increase as the eye fixation position moves further to the left.

These two types of neurons are segregated into the two sides of the brain. If the persistent activity in these neurons is due to reverberating interactions between them, we would expect positive firing-rate correlations between like-signed neurons and negative correlations between unlike-signed neurons. We are measuring correlations between neurons and investigating their sign and other characteristics.

Representation of Temporal Patterns in Humans

A. Penel, C. Hollweg, C.D. Brody

We have begun to investigate the question of temporal pattern representation using a very simple class of stimuli: 1-second-long patterns, each composed of three brief pulses of sound, with the first and the last pulse always 1 second apart. We will later use more complex stimuli, composed of more pulses. We have developed a task in which participants, after hearing such a three-pulse sequence, must indicate their estimate of the timing of the middle pulse by positioning a vertical line within a horizontal bar on a computer screen, i.e., participants have to translate into space what they perceived temporally. The timing of the second sound pulse varies randomly between trials. We have observed that subjects tend to categorize stimuli presented around 500 msec as being 500 msec (which corresponds to a subdivision of the 1-second interval into two equal intervals): middle pulse timings that were either slightly longer or slightly shorter than 500 msec were both reported as being closer to 500 msec than they actually were. In addition, participants were most consistent in their reponses for stimuli close to 500 msec and for those corrresponding to very short first or last intervals. Our hypothesis is that results obtained in this temporal-to-spatial task indicate tendencies in the subjects' temporal perception; we will validate this hypothesis by using the data to generate predictions about biases in more canonical temporal discrimination tasks. If correctly validated, we will then extend the method to more complex temporal patterns, of four or more pulses.

Influence of Rhythmic Grouping on Time Perception

A. Penel

Events in temporal patterns are not perceived independently from each other. Two perceptual organizational processes have been proposed: the extraction of a temporal regularity (of which the categorization at 500 msec for a 1-second interval in the previous project may be an example), and the sequential grouping of events into perceptual and mnemonic chunks, for example, according to temporal proximity. Previous work (Penel and Drake 2004) has suggested that the last short interval of such groups (preceding the long between-group interval) is perceived shorter than it is. This was demonstrated with musical sequences, professional musicians having to adjust (increase or decrease) all temporal intervals so that the sequences were perceived as temporally regular: This last short interval was adjusted longer than others. This needed to be replicated and further explored, however, using simpler sequences of identical tones and less musically trained participants. Indeed, one explanation for this perceptual bias is that it results from "musical" expectations about what is usually produced (performers usually slow down toward the end of groups). Our experiment confirmed previous data, and also showed a systematic lengthening of the already long between-groups interval, with no difference between nonmusicians and amateur musicians. These perceptual biases were observed at a fast tempo (300 msec), but not at a slow one (600 msec). This, together with the similarity of the results for professional, amateur musicians, and nonmusicians, suggests that the time perception effect is due to obligatory rhythmic grouping rather than musical expectations, as obligatory grouping is more likely to occur at a fast tempo than at a slow tempo. A critical test of the obligatory rhythmic grouping versus musical expectations origin of the effect will involve testing other sensory modalities (vision and touch). If the effect is due to musical expectations, it should not be observed there, but it should be observed if the phenomenon is purely temporal. Thus, we plan to conduct similar experiments using sequences of flashes and of mechanical impulses on the finger tip.

Finding Optimal Stimulus Ensembles

C. Machens [in collaboration with A. Herz, Berlin]

A central aim of theoretical neuroscience is to develop general principles that explain the processing of stimuli in sensory systems. One such principle has been formulated in the Efficient Coding Hypothesis, which states that sensory neurons strive to represent infor-

mation about natural stimuli as efficiently as possible. However, since different stimuli come with different behavioral relevance, the representational capacity of sensory neurons might be biased toward the more relevant stimuli. In a collaboration with the group of Andreas Herz, Berlin, we have analyzed this potential bias in grasshopper auditory receptor neurons. The optimal ensemble of stimuli was searched for using on-line electrophysiological experiments. Although grasshopper songs are well-mapped by the auditory receptor neurons, there is no one-to-one correspondence with the optimal stimulus ensemble. Rather, our results show that specific aspects of the songs (such as gaps or syllable onsets and offsets) are encoded better, suggesting that the system is more selective at its earliest stage than previously thought.

Linearity of Receptive Fields in the Auditory Cortex

C. Machens [in collaboration with A. Zador, Cold Spring Harbor Laboratory]

Despite years of research, the auditory cortex remains elusive territory. Apart from its basic tonotopology, not much is understood about its computational properties. Based on recordings with simple artificial sounds, several recent studies have proposed that cortical neurons decompose sounds into a set of simple, linear receptive fields. However, in collaboration with Mike Wehr and Anthony Zador here at CSHL, we have shown that the success of the linear receptive field largely depends on the kind of stimuli used. Focusing on natural sounds, i.e., the sounds that the auditory cortex is built to process, we found that cortical responses often cannot be predicted by the linear receptive field model. To understand the nonlinear response properties of cortical neurons, we have studied several enhancements of the basic linear model. Including some simple nonlinearities in the model, we could show that the failure of the model cannot be attributed to rectification, saturation, or adaptation to mean intensity. Rather, the highly nonlinear response properties of auditory cortical neurons must be attributable to nonlinear interactions between sound frequencies and time-varying properties of the neural encoder.

Deriving Spike Timing Learning Rules

C.D. Brody [in collaboration with J. Hopfield, Princeton University]

Plasticity in connections between neurons allows learning and adaptation, but it also allows noise to degrade the function of a network. Ongoing network self-repair is thus necessary. We describe a method to derive spike-timing-dependent plasticity rules for self-repair, based on the firing patterns of a functioning network. These plasticity rules for self-repair also provide the basis for unsupervised learning of new tasks. The particular plasticity rule derived for a network depends on the network and task. Here, self-repair is illustrated for a model of the mammalian olfactory system in which the computational task is that of odor recognition. In this olfactory example, the derived rule has qualitative similarity with experimental results seen in spike-timing-dependent plasticity. Unsupervised learning of new tasks by using the derived self-repair rule is demonstrated by learning to recognize new odors.

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PRINCIPLES OF BRAIN DESIGN

D.B. Chklovskii B. Chen A. Stepanyants
S. Song Q. Wen

Understanding brain function requires unraveling synaptic connectivity in neuronal circuits. Con-sequently, the main goal of our laboratory is to help assemble the brain wiring diagram. We pursue this ambitious goal from several directions. First, we analyze physical constraints on brain design and function in order to determine "engineering" principles that governed brain evolution. These principles, like the laws of conservation in physics, can narrow down the set of possible circuits and help focus our efforts in the search for the models of brain function. Second, we assemble a probabilistic description of neuronal circuits based on light microscopy data. Although light microscopy does not establish synaptic connectivity conclusively, it is, currently, the only feasible approach to complex neuronal networks, such as the cortical column. Third, we are developing an algorithm for automatic reconstruction of the brain wiring diagram from serial section electron microscopy. This may be the most promising approach to describing smaller networks, such as invertebrate brains or smaller subsets of vertebrate nervous system. In addition, we are analyzing assembled wiring diagrams to make a connection between anatomy and behavior. In 2003, we made excellent progress in all of these research directions.

Assembling the Complete Wiring Diagram of the *C. elegans* Nervous System

B.L. Chen, D.B. Chklovskii [in collaboration with D. Hall, Albert Einstein College of Medicine]

Despite common claims to the contrary, the full wiring diagram for the *Caenorhabditis elegans* nervous system has not been assembled. We take advantage of existing and newly obtained electron microscopy photographs to complete the project started 30 years ago by S. Brenner's group at Cambridge. Completion of the project will provide a unique data set, which can be used to understand the function of neuronal networks. In addition to recording neuronal pairs making synaptic connections, we also tabulate the location of individual synapses.

Why Are Most Neurons in the Head?

B.L. Chen, D.B. Chklovskii

We pursue the hypothesis that the placement of neurons in an animal minimizes wiring costs for given functional constraints. Using the available wiring diagram of the *C. elegans* nervous system, we solve for the optimal layout of its nonpharyngeal neurons. Rather than enumerating an exponentially large number of placements, we use a quadratic cost function that can be solved exactly. The solution demonstrates that (1) neurons in the same ganglion cluster together, (2) the calculated ordering of neurons follows actual anterior to posterior order, and (3) the predicted coordinates of neurons within the worm resemble their actual locations. In addition, we find that rare neurons deviate strongly from our predictions, suggesting the existence of other yet unknown functional constraints. These results not only prove the importance of wiring cost minimization in neuronal placement, but also spotlight neurons whose functional role is not captured simply by the wiring diagram.

Synaptic Connectivity and Neuronal Morphology: Two Sides of the Same Coin

D.B. Chklovskii

Among the cells of the body, neurons stand out by the intricate shape of cell processes, or axonal and dendritic arbors. What is the functional role of these processes? To answer this question, I considered wiring up a large highly interconnected neuronal network, such as a cortical column. Implementation of such network in the allotted volume requires all of the salient features of neuronal morphology: the existence of dendrites as well as axons, their branching, and the existence of dendritic spines. Therefore, these features are adaptations that make a large highly interconnected network possible. Moreover, actual dimensions of axons and dendrites are highly optimized, suggesting that high interconnectivity is essential for cortical function.

To Myelinate or Not to Myelinate?

Q. Wen, D.B. Chklovskii

Myelinated and nonmyelinated axons coexist in the brain. What determines whether a given axon is myelinated or not? Rushton argued that, for a given conduction delay, the thinner axon is chosen from myelinated and nonmyelinated alternatives. If the required propagation speed is below critical value, nonmyelinated axons are thinner. Otherwise, myelinated axons should be used. This argument leads to the prediction of a critical axon diameter, which segregates myelinated and nonmyelinated axons. Since the critical diameter is not observed experimentally, we decided to revisit the issue. We show that different requirements on conduction delay do not necessarily lead to the critical diameter. By considering two optimization scenarios, we show that the appearance of an axon diameter spectrum depends on the relative cost per volume between myelinated and nonmyelinated axons. Both scenarios suggest that if cost per volume of myelinated axons is significantly less than that of nonmyelinated axons, there would be a gap between the distributions of nonmyelinated and myelinated axon diameters. If myelinated axons are costlier than nonmyelinated, the diameter distributions would overlap. In addition, the second scenario predicts a gap in the conduction velocity spectrum, independent of the relative cost. By comparing our predictions with data from the corpus callosum, which shows overlap of the diameter distributions, we conclude that myelinated axons are costlier than nonmyelinated axons. Prediction of a gap in the conduction velocity spectrum needs to be tested experimentally. In conclusion, distribution of diameters for myelinated and nonmyelinated axons depends on the relative cost of myelination. Overlap between the two distributions in the corpus callosum suggests that myelinated axons are costlier than nonmyelinated axons.

Understanding Segregation of the Cortical Neuronal Network into Gray and White Matter

Q. Wen, D.B. Chklovskii

A ubiquitous feature of the mammalian cerebral cortex is the segregation of gray matter, containing mostly local circuits, and white matter, containing mostly long-range myelinated axons. Why does such segregation occur and why is the gray matter confined to a narrow sheet on the surface of the cortex? We propose that the observed architecture is the result of maximizing neuronal connectivity while minimizing conduction time delays. We start from building a neuronal network roughly corresponding to the cortical column. This network contains k neurons with all-to-all connectivity. Conduction time delay increases with k and limits the number of neurons in a fast-functioning highly interconnected network. To construct a larger fast neuronal network containing neurons, it is necessary to give up the all-to-all connectivity and use a small-world network architecture. In this architecture, each neuron connects with k local neurons and sends *one* long-range myelinated axon to another locale. We determined optimal parameters for this model of the cortical network.

Search for Motifs in Mammalian Neocortex

D.B. Chklovskii, M. Reigl, S. Song [in collaboration with J. Sjostrom and S. Nelson, Brandeis University]

In the quest for basic rules of synaptic connectivity, we measure statistics of connections among layer-5 pyramidal neurons from the rat visual cortex. Connectivity was established electrophysiologically in several hundred simultaneous quadruple whole-cell recordings. First, we determined the probability for a pair of neurons to be unconnected –83%, unidirectionally connected –12%, bidirectionally connected –5%. These results confirm previous reports that bidirectional connections are overrepresented relative to the expectations based on the independence of synaptic connections. Second, we determined the probability of encountering triplets belonging to each of the 16 possible connectivity classes and compared it with the theoretical predictions based on the doublet connectivity statistics. We found several classes with significant deviations in the numbers of triplets. Most of the deviations fit the following rule: Triplets with all-to-all connections (either unidirectional or bidirectional) are overrepresented by several hundred percent. Assayed neurons were always within 100 μm of each other, thus assuring significant overlap between their arbors. Therefore, the locality of connectivity cannot account for this rule. In conclusion, connectivity among layer-5 pyramidals is inhomogeneous: All-to-all connected triplets and bidirectional pairs are highly overrepresented. This may reflect the action of learning rules in the course of development, which could be inferred by analyzing the statistics of synaptic strength.

Optimal Stimulus Design

D.B. Chklovskii, A. Stepanyants

Neurons in the brain respond to sensory stimuli by firing action potentials. The firing rate of a particular neuron depends on which stimulus is presented to the animal. Stimulus to which a neuron responds with the highest firing rate is called the optimal stimulus. Therefore, a neuron can be thought of as a detector for an optimal stimulus. Although we know what the optimal stimulus is for some neurons, for majority of neurons, it remains unknown. Our goal is to design a computer algorithm that would perform a search for an optimal stimulus automatically. This is a very difficult task because the dimensionality of the stimulus space is extremely high. Currently, we are designing an appropriate algorithm and testing it against a computer model of a neuron with a known optimal stimulus.

Domains of Potential Connectivity of Cortical Neurons

A. Stepanyants, D.B. Chklovskii [in collaboration with J. Hirsch, University of Southern California; Z. Kisvarday, Bohum]

Synaptic connectivity among cortical neurons may vary with time due to the growth and retraction of dendritic spines. This suggests that the invariant description of cortical circuits should be formulated on the level of potential synapses, i.e., locations in the neuropil where an axon of one neuron is present within spine length (~2 μm) from a dendrite of another. The number of potential synapses between two neurons can be determined from the overlap region of their dendritic and axonal arbors and branch densities. On the basis of three-dimensional reconstructions of axonal and dendritic arbors, we evaluated the expected number of potential synapses among spiny neurons from the cat visual cortex. Typically, any two neurons with significant arbor overlap are potentially connected, i.e., have at least one potential synapse. For spiny neurons from different cortical layers, we compute local domains, which contain potentially connected spiny neurons. These columnar domains may be an essential element of the cortical architecture. Their size is in between the minicolumns (Peters and Payne, *Cerebral Cortex 3:* 69–78 [1993]) and the hypercolumns (Hubel and Wiesel, *Proc. R. Soc. Lond. B. 198:* 1–59 [1977]). In these domains, connections between most spiny neurons can be implemented by local synaptogenesis.

Relationship between Potential and Actual Synapses

A. Stepanyants, D.B. Chklovskii [in collaboration with A.M. Thomson, University of London]

To take advantage of potential connectivity calculations in unraveling the function of cortical circuits, it is important to establish a link between potential and actual connectivity. We are testing a hypothesis that a pair of synaptically connected excitatory neurons has a synapse at all potential synaptic sites. In other words, connectivity between excitatory neurons is "all or none," where if two neurons connect, they convert all their potential synapses into actual ones. We use the Neurolucida system to trace pairs of synaptically connected neurons (determined through electrophysiology), identify all potential synaptic sights, and look for the presence of spines and/or boutons at these locations.

Mechanisms of Subcellular Domain Specificity in GABAergic Neurons

A. Stepanyants [in collaboration with J.Z. Huang, Cold Spring Harbor Laboratory]

Axons of GABAergic basket cells exhibit affinity toward the somata of pyramidal cells. We help to parse developmental mechanisms leading to such affinity by analyzing the time course of axonal trajectories. The Huang lab here at CSHL has developed an ideal preparation for investigating this question. By using cell-type-specific promoters and BAC (bacterial artificial chromosome) engineering, they are able to visualize different GABAergic interneurons and postsynaptic pyramidal cells in transgenic mice and in organotypic cortical slice cultures. Confocal images of these systems are being collected for different developmental stages. We are in the process of analyzing these images by using our newly developed correlation analysis. This approach will provide us with the developmental time course of correlations between axons of basket cells and pyramidal somata. By comparing this time course with the time course of morphological changes of the cells, we should be able to identify developmental mechanisms involved in the formation of these correlations.

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BRAIN DEVELOPMENT AND PLASTICITY

H.T. Cline C. Aizenman, C. Akerman, J. Bestman, K. Bronson, K. Burgos, S.-L. Chiu, R. Ewald, K. Haas, A. Javaherian, K. Jensen, K. John, V. Thirumalai, E. Rial Verde, E. Ruthazer, P. Sharma

Sensory input into the brain is essential for organizing brain connectivity and circuit function during development and for modifying neuronal circuits with learning in the mature nervous system. My lab is working to determine the cellular and molecular mechanisms that operate to establish and modify brain connections during development. Nervous system dysfunction may arise from failure of these mechanisms to operate during development or in the mature nervous system. We address this issue by examining the structural and functional development of the visual system in amphibian tadpoles. These animals are transparent, which allows us to observe directly the development of the brain in living animals. In addition, we assess neuronal function using electrophysiological assays of synaptic connectivity and synaptic plasticity. We combine these studies with gene transfer methods, which allow us to test the function of the genes of interest in brain development. One of the highlights of our research this year was the demonstration that glutamate receptor activity following visual stimulation is required for the normal development of topographically organized connections from the eye to the central nervous system. This study lays the groundwork for future work intended to identify specific mechanisms by which brain connectivity develops in the intact animal. Given the high degree of conservation of mechanisms related to brain development and plasticity, our work will identify key regulatory mechanisms governing brain plasticity across species. Below are highlighted a few projects carried out in the laboratory during the last year.

STRUCTURAL DEVELOPMENT OF NEURONS USING TIME-LAPSE IN VIVO IMAGING

Excitatory and inhibitory interneurons have a key role in the establishment of neuronal responses and circuit properties in the brain. Despite their importance in brain function, the structural development of interneurons has not been well studied. We used in vivo time-lapse imaging in intact anesthetized *Xenopus* tadpoles to determine the morphological events underlying the development of interneuron dendritic arbor structure. Single optic tectal neurons were labeled with DiI and imaged at daily intervals over 4 days in intact albino *Xenopus* tadpoles. The same neurons were also imaged at shorter intervals to determine the dynamic rearrangements in arbor branches that accompany large-scale arbor growth. Tectal interneurons, like projection neurons, develop from neuroepithelial cells located near the ventricular layer. They elaborate complex dendritic arbors over a period of 2 days. Short-interval time-lapse images reveal that tectal interneuron arbors have rapid rates of branch additions and

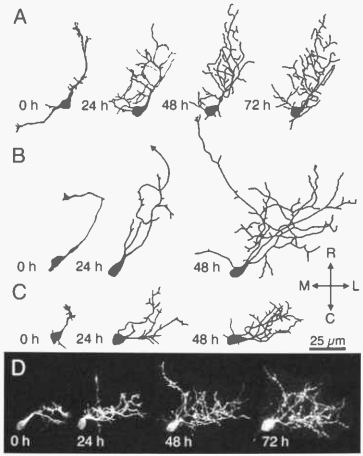

FIGURE 1 In vivo time-lapse images of optic tectal neurons collected at the intervals stated. Note the variety of patterns of arbor development and final structure of local interneurons within the *Xenopus* optic tectum. We are interested in determining the cellular and molecular mechanisms that control the development of dendritic arbors. These mechanisms will in turn control the development of neuronal circuits with receive and process visual information.

retractions. We identified four patterns of interneuron arbor development, based on the cell morphology and types of structural rearrangements that occur over the development of the neuronal arbor. A surprising feature of interneuronal development is the large extent of structural rearrangements: Many interneurons extend transient processes so that the neuronal structure is dramatically different from one day to the next. Because the majority of synaptic contacts are formed on dendrites, the structural changes we observed in interneuronal dendritic arbors suggest that optic tectal circuits are extremely plastic during early stages of development.

ACTIVITY-DEPENDENT MODIFICATIONS OF VISUAL RESPONSIVENESS IN VIVO

Carlos Aizenman carried out a series of electrophysiology experiments testing the effect of visual stimulation on retinotectal synaptic responses. We find that glutamatergic synaptic responses are regulated in an activity-dependent manner by intracellular poly-amines. Subsequent experiments demonstrated that visual activity also affects tectal cell excitability. This increase is correlated with enhanced voltage-gated Na^+ currents. Because the same visual stimulation protocol also induces a polyamine synthesis-dependent reduction in Ca^{++}-permeable, AMPA-R (α-2-hydroxy-5-methyl-4-isoazole receptor)-mediated synaptic drive, the increased excitability may compensate for this reduction. Accordingly, the change in excitability was prevented by blocking polyamine synthesis during visual stimulation and was rescued when Ca^{++}-permeable AMPA-R-mediated transmission was selectively reduced. Colin Akerman and Carlos Aizenman found that these changes in excitability also rendered tectal cells more responsive to synaptic burst stimuli, improving visual stimulus detection. The synaptic and intrinsic adaptations function together to keep tectal neurons within a constant operating range while making the intact visual system less responsive to background activity, yet more sensitive to burst stimuli.

STRUCTURAL DYNAMICS IN RETINAL AXONS ARE REGULATED BY CORRELATED ACTIVITY

A requirement for patterned visual activity during visual system development has been demonstrated in a number of experimental systems; however, it was not clear whether afferent coactivity had a role in topographic map formation at early stages of development. To address this question, Ed Ruthazer resurrected the dually innervated tectum preparation in albino *Xenopus*. Unilateral tectal ablation in young tadpoles results in the convergence of retinal inputs from both eyes onto the remaining tectal lobe. Initially, the inputs from the two eyes are largely overlapping, and they gradually sort out into eye-specific zones. This experimental system allowed Ed to test whether afferent coactivity, quantified as the relative intensity of labeled inputs from one eye compared to the other, has a role in the axon arbor rearrangements that contribute to the eye-specific segregation of the inputs. Ed imaged single dye-labeled retinal axons at 2-hour intervals over 8 hours and mapped the distribution of branches that were added and retracted from the arbor over the 8-hour period. At the end of the imaging session, retinal inputs from both eyes were bulk-labeled with different colors. Dual imaging of the inputs from both eyes provided a quantitative measure of the degree of convergence of inputs from the two eyes, and this was taken as a measure of afferent coactivity. We found that axon branches are added independent of the degree of innervation by one eye or the other, but that branch stabilization was significantly increased in regions that were innervated by axons originating from the same eye as the imaged axon. This suggested that afferent coactivity was a key factor in branch stabilization. To test this, we exposed animals to MK801, the NMDA-R (*N*-methyl-D-aspartate receptor) antagonist. Interestingly, MK801 blocked the selective elimination of branches in territory dominated by the opposite eye, suggesting NMDA-R-dependent branch elimination from noncoactive sites. This mechanism, combined with NMDA-R-dependent selective stabilization of branches in coactive regions would result in the formation of topographic maps.

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GENETICS OF MEMORY IN *DROSOPHILA*

J. Dubnau A. Blum
T.W. Jung

The long-term goal of our research is to understand memory. Dissection of complex behaviors such as memory and learning will require a multidisciplinary approach that will include discovery and manipulation of the relevant genetic, cellular, and anatomical pathways, as well as computational modeling of how information is processed in the brain. Work in genetic model systems such as *Drosophila* can contribute to our understanding in two main ways. First, by enabling the discovery of genes and genetic pathways underlying behavior, genetic model systems provide entry points for subsequent interventionist experiments. Second, systematic manipulation of gene function in relevant anatomical loci of the brain allows a conceptual integration of findings from cellular and behavioral neuroscience.

We use the *Drosophila* model system, which offers an economy of scale to discover and develop hypotheses in a relatively cost-effective and rapid manner. Then, given the remarkable evolutionary conservation of genetic, cellular, and behavioral functions, these hypotheses can be pursued in mammalian model systems.

Gene Discovery with DNA Microarrays

J. Dubnau [in collaboration with A.S. Chiang, Taiwan, and J. Barditch, L. Grady, F. Bolduc, and T. Tully, Cold Spring Harbor Laboratory]

In collaboration with the lab of T. Tully here at CSHL, we have used a combination of behaviorally specific training protocols and expression profiling with DNA chips to identify transcriptional responses during memory consolidation. We have used this approach to compare gene expression profiles after spaced training, which induces both short-term and protein-synthesis-dependent long-term memory, and massed training, which only induces short-lived memory. We have identified a large number of candidate memory genes differentially expressed at three different retention intervals after spaced versus massed training. Using real-time polymerase chain reaction (PCR) follow-up assays, and an independent microarray, we have confirmed differential expression for 42 of these candidates, establishing the validity of this approach.

Ultimate confirmation that a gene is involved in memory formation rests with the demonstration that in vivo modulation (disruption) of the gene alters that process with some specificity. With that aim in mind, we are focusing on local translational control, one of several pathways suggested from the array experiments. Our genetic studies already support a role in memory for several components of this pathway. These include *staufen* and *oskar*—known components of a cellular mRNA localization machinery, *pumilio*—which is a translational repressor protein, and *thor*, which encodes a fly homolog of 4EBP, a translational regulator.

We now are using existing molecular genetic reagents to investigate the role of this pathway in memory and to visualize local translation in neurons of the adult brain.

Genetic Dissection of Anesthesia-resistant Memory

A. Blum, J. Dubnau [in collaboration with A.S. Chiang and Y.S. Chen, Taiwan; J. Barditch, S.L. Chiu, M. Regulski, and T. Tully, Cold Spring Harbor Laboratory; and J.D. Armstrong, Edinburgh]

In both vertebrate and invertebrate animals, anesthetic agents cause retrograde amnesia for recently experienced events. In contrast, older memories are resistant to the same treatments. In *Drosophila*, anesthesia-resistant memory (ARM) and long-term memory (LTM) are genetically distinct forms of long-loved memory that exist in parallel for at least a day after training. ARM is disrupted in *radish* mutants but is normal in transgenic flies overexpressing a CREB (cAMP response-element-binding protein) repressor transgene. In contrast, LTM is normal in *radish*

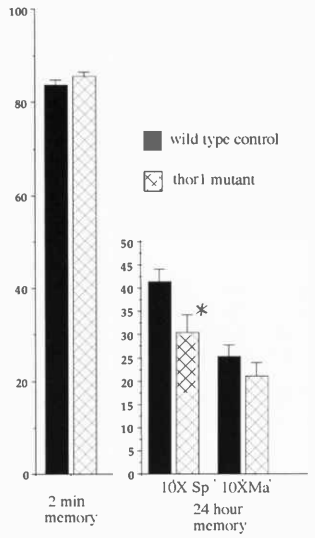


FIGURE 1 *thor* is a long-term memory mutant. *thor* mutant animals display normal levels of memory measured 2 minutes after a single training session. In contrast, *thor1* mutants exhibit defective long-term memory. Memory is reduced in *thor1* mutants when measured 24 hours after ten sessions of spaced training (20XSp), but not after ten sessions of massed training (10XMa).

mutants but is disrupted in CREB repressor transgenic flies. Until now, nothing was known about the molecular, genetic, or cellular biological pathways underlying ARM. We have identified *radish* as a phospholipase-A2, providing the first clue about signaling pathways underlying ARM in any animal. An enhancer-trap allele of *radish* (C133) also provides an entry point to study the anatomical circuits underlying ARM. rsh^{C133} driven reporter expression does not label mushroom bodies, which are the primary anatomical focus of olfactory memory research in *Drosophila*. Instead, it reveals a novel anatomical pathway. Transgenic expression of PLA2 in this pattern restores normal levels of ARM to *radish* mutants, whereas transient disruption of neural activity in rsh^{C133} neurons inhibits memory retention. Identification of *radish* as a phospholipase-A2 and the neural expression pattern of this enhancer trap significantly broaden our understanding of the biochemistry and anatomy underlying olfactory memory in *Drosophila*. These findings also are consistent with pharmacological studies of PLA2 signaling in memory formation in vertebrate animals.

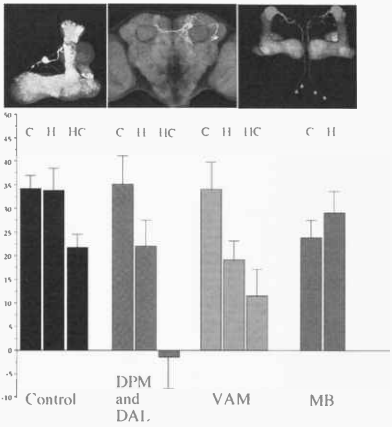
Reversible Disruption of Synaptic Transmission

J. Dubnau [in collaboration with A.S. Chiang, Taiwan]

We are using a temperature-sensitive dynamin transgene, which disrupts synaptic transmission reversibly and on the time scale of minutes, to investigate the temporal requirements for ongoing neural activity during learning, memory consolidation/storage, and retrieval. We are using a panel of Gal4 enhancer drivers to focus expression of this dynamin transgene within specific subpopulations of neurons. With this approach, we are now able to reversibly silence reproducibly specific groups of neurons in vivo. This strategy permits a dissection of the anatomical circuitry underlying memory. We already have demonstrated a role for synaptic transmission in mushroom body neurons during memory retrieval, but surprisingly not during acquisition or storage of early memory. These data suggest that the synaptic plasticity underlying olfactory associative learning reside in mushroom body dendrites and/or upstream of the mushroom body and that resulting alterations in synaptic strength modulate mushroom body output during memory retrieval.

More recently, we have used a series of Gal4 enhancer lines identified by transposon insertion in memory genes to spatially restrict the dynamin block to additional functionally relevant subcomponents of the memory circuit. We have demonstrated an anatomical dissection of the requirements for neural activity during formation and storage of anesthesia-sensitive versus anesthesia-resistant memory (Fig. 2). We now are extending this approach to a panel of Gal4 driver lines that yield expression in additional neuronal populations.

FIGURE 2 Anatomical dissection of anesthesia-sensitive and anesthesia-resistant memory (ARM). (*Top panels*) GFP reporter expression in several neurons that participate in the circuitry underlying olfactory memory. (*Left panel*) Dorsal paired medial neuron massively innervates the axonal outputs of the mushroom bodies. (*Middle panel*) Dorsal-anterior-lateral neuron sends fibers into parts of the posterior superior protocerebrum, including mushroom body dendritic field. (*Right panel*) Ventral-anterior-medial neurons innervate the alpha lobes of the mushroom bodies. (*Bottom panel*) Expression of the temperature-sensitive dynamin transgene under control of Gal4 drivers permits focused transient inhibition of dynamin-dependent activity in specific neuronal subtypes. Three Gal4 lines were used: one which expresses in DPM and DAL, one which expresses in VAM, and a third which expresses in mushroom bodies. In the case of the DPM and DAL line as well as the VAM line, transient heating to the restriction temperature (*H*) causes a partial amnesia measured 3 hours after training (compare control treatment [*C*] with heat shifted [*H*]). In contrast, this heat shift has no effect on a control strain or on a line that expresses the dynamin transgene in mushroom bodies. Application of a cold-shock anesthesia cold-shock treatment completely erases anesthesia-sensitive memory, providing a pure measure of ARM. Use of cold shock in combination with heat-shift (HC) reveals an anatomical dissection of anesthesia-sensitive memory from anesthesia-resistant memory. In the DPM/DAL-expressing animals, this combination treatment erases all memory, suggesting that the memory disrupted by dunamin alone (*H*) is ARM. In contrast, the combined treatment in VAM neurons reveals that a significant portion of the memory remaining after the dynamin effect is ARM. Thus, inhibition of VAM-dynamin mostly disrupts anesthesia-sensitive memory.



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SIGNAL TRANSDUCTION, STEM CELLS, AND DIFFERENTIATION

G. Enikolopov J. Encinas, J. Hemish, B. Kuzin, M. Maletic-Savatic, T. Michurina, B. Mish, N. Peunova, K. Ravi, J. Roig, V. Scheinker

Stem and progenitor cells receive specific cues that control their self-renewal and their transition through differentiation cascades. Our laboratory is interested in stem cells in the adult organism and the signaling molecules that control the division of stem and progenitor cells and guide them toward their differentiated fate. Our efforts have been focused on the versatile signaling molecule nitric oxide (NO). NO has a wide range of effects in humans, from regulating blood pressure to modulating brain activity. We have discovered that NO has yet another crucial role: It regulates division of stem and progenitor cells and mediates their progression through differentiation cascades. Part of this regulation is due to the antiproliferative properties of NO: Division of stem and progenitor cells can be manipulated by supplying or removing the source of NO. For instance, we have found that NO acts as an important negative regulator of cell division in the adult brain, such that it is possible to increase the number of neural stem cells by suppressing the production of NO in the brain.

This year, we have been focusing on the molecular interactions of NO with key regulatory pathways in the cells, particularly those that control cell division. Using transcriptional profiling as a test, we have identified several signaling pathways that are required for the NO signal to induce gene expression and cell cycle arrest. We have also shown that NO mediates the homeostasis of hematopoietic stem cells in the bone marrow. Finally, we have generated several animal models to study neural stem cells and the action of NO on adult neurogenesis.

NO Is an Important Negative Regulator of Neurogenesis in the Adult Brain

M. Packer, Y. Stasiv, J.M. Encinas [in collaboration with S. Goldman, Cornell Medical School, and H. Westphal, National Institutes of Health]

The vast majority of neurons in the mammalian brain are produced during embryonic development. However, there are several restricted zones in the adult brain where new neurons are constantly generated from a resident population of neural stem and progenitor cells. At the same time, it is possible to detect neural progenitors throughout the adult nervous system (e.g., in ex vivo experiments), suggesting the existence of negative regulators of neurogenesis in the adult brain. None of the postulated negative regulators have been identified so far in the adult nervous system. We found that NO is used as a negative regulator to control the generation of new cells in the neurogenic areas of the adult brain. In a pharmacological approach, we suppressed production of NO by introducing inhibitors of NO synthase (NOS) into the ventricles of the adult brain. In a genetic approach, we generated a null mutant for the neuronal NOS gene. In both models, the number of new cells generated in neurogenic areas, the olfactory subependyma and the dentate gyrus, was strongly augmented. Importantly, increased proliferation of progenitor cells did not alter the fate of the extra cells. Our results reveal a role for NO as an essential regulator of neural stem/progenitor cells in the adult brain. They also suggest a strategy for enhancing neurogenesis in the damaged central nervous system. Our current efforts are to determine the subclass of stem/progenitor cells that is controlled by NO in the adult brain.

NO Is a Regulator of Hematopoietic Stem Cell Activity

T. Michurina, P. Krasnov [in collaboration with R.C. Mulligan, Harvard Medical School]

Hematopoietic stem cells give rise to various multipotent progenitor populations, which expand in response to cytokines and which ultimately generate all of the elements of the blood. We found that it is possible to increase the number of stem and progenitor cells in the bone marrow by suppressing the activity of NOS. Exposure of mice to NOS inhibitors either directly or after irradiation and bone marrow transplantation increases the number of stem cells in the bone marrow.

In the transplantation model, this increase is followed by a transient increase in the number of neutrophils in the peripheral blood. Thus, our results indicate that NO is important for the control of hematopoietic stem cells in the bone marrow. They further suggest that suppression of NO synthase activity may allow expansion of the number of hematopoietic stem and progenitor cells for therapeutic purposes.

NO Regulates Cell Proliferation and Cell Motility in Early *Xenopus* Development

N. Peunova, V. Scheinker

NO has been implicated in cell differentiation and organism development. However, the interconnection between NO signaling and the major developmental pathways remains unknown. We investigated the role of NO in early *Xenopus* development using both gain-of-function and loss-of-function approaches. We examined changes in embryonic development after microinjecting either mRNA encoding *Xenopus* NOS (XNOS1) or the NO donor SNAP; conversely, we blocked NOS activity by injecting either mRNA encoding a dominant-negative form of the protein, dnXNOS, or chemical inhibitors of NOS. Excess NO production decreases cell proliferation in the embryo. In contrast, deficits in NO production result in excessive cell proliferation and distort cell motility. Inhibition of NOS activity prevents normal axis extension and neural tube closure during gastrulation and neurulation. Thus, at early stages of embryonic development, NO coordinates cell proliferation and morphogenetic cell movement. A deficit of NO also leads to profound defects in organogenesis. Further investigation of the signaling pathways affected by NO in the *Xenopus* embryo points to a critical role of small GTPases in the function of NO during development. Using specific inhibitors, we have identified molecular targets and pathways used by NO to control cell cycle and cell motility during embryonic development.

NO and *Drosophila* Development

B. Kuzin, Y. Stasiv, V. Scheinker [in collaboration with M. Regulski and T. Tully, Cold Spring Harbor Laboratory]

NO is an essential regulator of *Drosophila* development and physiology: It has been implicated in visual system development, immunity, behavior, response to hypoxia, and regulation of cell cycle progression during development. We found a novel mode of regulation of NOS function that employs endogenously produced truncated protein isoforms of *Drosophila* NOS (DNOS). These isoforms inhibit NOS enzymatic activity in vitro and in vivo, reflecting their ability to form complexes with the full-length DNOS protein. Truncated isoforms suppress the antiproliferative action of DNOS1 in the eye imaginal disc by impacting the retinoblastoma-dependent pathway, yielding hyperproliferative phenotypes in pupae and adult flies. Our results indicate that endogenous products of the DNOS locus act as dominant-negative regulators of NOS activity during *Drosophila* development. They also point to a novel mechanism for the regulation of NO production in animal cells.

In a related study, Michael Regulski carried out a wide screen for lethal DNOS mutants and demonstrated an essential function for NO during early *Drosophila* development. He now sequences a panel of mutant DNOS genes to position the mutations and thus provide insights into the crucial sites in the DNOS protein molecule.

Mechanisms of NO Action

J. Hemish, N. Nakaya, K. Ravi [in collaboration with V. Mittal, Cold Spring Harbor Laboratory]

One approach to reveal the targets of NO action is to identify the genes that are activated by NO and to deduce the pathways that NO uses to activate transcription. We employed transcriptional profiling using microarrays to determined the temporal order of gene activation induced by NO. We then used that information to gain insights about the signaling pathways that mediate the action of NO: Applying inhibitors of particular signaling pathways, and using cells from animals with a deleted *p53* gene, we were able to define groups of genes that require PI3 kinase, protein kinase C, NF-κB, p53, or their combination, for activation by NO. Our results demonstrate that NO utilizes several independent signaling pathways to induce gene expression.

Having determined several major signaling cascades that are activated by NO, we are now trying to determine whether NO can directly modify the crucial members of these pathways by *S*-nitrosylation. Furthermore, we used cells from a panel of mouse mutants to show that NO affects several independent

checkpoints to induce cell cycle arrest. Finally, we study the action of a novel NO-inducible gene, *noxin*, that we have identified in the screen for NO-inducible genes. We have generated a knockout mutant of *noxin* and are now investigating whether *noxin* serves as a link between NO signaling and the cell cycle control.

Neural Stem Cells in the Adult Nervous System

J. Mignone, M. Maletic-Savatic, A. Vaahtokari, J. Roig, B. Mish, C. Matthews [in collaboration with A.-S. Chiang, Tsing Hua University, Taiwan; D. Steindler, University of Florida; R. Hoffman, AntiCancer; and S.Y. Kim, Cold Spring Harbor Laboratory]

To follow the transition of neural stem and progenitor cells along the differentiation cascades, we generated a series of transgenic reporter mouse lines. In several of them, neural stem cells of the embryonic and adult brain are marked by the expression of green, cyan, and red fluorescent proteins (GFP, CFP, RFP), expressed under the control of the *nestin* gene regulatory elements. Stem and progenitor cells can be isolated from these animals with high purity using FACS (fluorescence-activated cell sorter) and can generate multipotential neurospheres. In the adult brain of *nestin*-GFP animals, GFP-expressing cells are more than 1400-fold more efficient in generating neurospheres than GFP-negative cells and, despite their small number, give rise to 70 times more neurospheres than the GFP-negative population; thus, our data show that *nestin*-GFP-expressing cells encompass the majority of the neural stem cells in the adult brain. These cells can be divided into two groups which represent distinct classes of neuronal precursors in the adult brain and may reflect different stages of neuronal differentiation.

We also found that in some tissues of our model animals, GFP expression may mark stem/progenitor cells for these tissues. For instance, we found that *nestin*-GFP cells can be found in the bulge region of the hair follicle, where stem cells for the follicle are thought to be located. By a number of criteria, these cells indeed behave as progenitor population of the follicle outer-root sheath; in addition, these cells can generate progeny of diverse lineage, including neuronal. We now study the ability of *nestin*-expressing cells from non-neural tissues to reveal their neural potential both in vitro and in vivo, after transplantation into adult mouse brain.

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CONSTRUCTION AND PLASTICITY OF GABAERGIC CIRCUITS

Z.J. Huang F. Ango, B. Chattopadhyaya, G. diCristo, S. Kulhman, T. Pal, Q. Wang, C. Wu, H. Yang

My laboratory is studying the assembly, plasticity, and function of the GABAergic inhibitory network in the brain. The GABAergic network consists of a rich array of cell types with distinct morphology, physiological properties, and synaptic connectivity patterns and may constitute a scaffold that organizes local neuronal connectivity and activity. The "design" and function of the GABAergic system are strikingly different from that of the glutamatergic excitatory system. Increasing evidence suggests that GABAergic transmission is particularly effective in regulating the precise timing of electrical signaling both within a principal neuron and among populations of principal neurons. Networks of GABAergic interneurons have been hypothesized to entrain populations of principal neurons and thus impose a coordinated "context" for the "content" carried by networks of principal neurons during perception, cognition, movement control, learning, and memory. Aberrant GABAergic functions have been implicated in devastating neurological and psychiatric disorders such as epilepsy and schizophrenia. The diverse and distinct GABAergic functions result in part from the pattern of GABAergic connectivity. A prominent feature of GABAergic connectivity is the targeting of different classes of GABAergic synapses to subcellular compartments of principal neurons (spines, dendrites, soma, and axon hillock). Our understanding of the construction and plasticity of GABAergic circuits has thus far been hampered by their complexity and heterogeneity.

Progress in our laboratory in the past year is beginning to define the logic and a sketch of the construction of GABAergic circuits in the mammalian central nervous system. First, by combining genetic engineering and high-resolution imaging, we have established both in vivo and in vitro methods to visualize and quantify the targeting of specific classes of GABAergic synapses onto restricted subcellular compartments of principal neurons. We discovered that subcellular targeting of GABAergic synapses in the primary visual cortex does not require instructions from sensory input and thus is largely guided by genetically encoded mechanisms and experience-independent forms of neuronal activity. Second, we are beginning to define the molecular signals underlying subcellular synapse targeting. We discovered that the ankyrin-G-based membrane cytoskeleton is the key molecular machinery that, through subcellular recruitment of the L1 family immunoglobulin cell adhesion molecules (CAMs), directs GABAergic innervation to the axon hillock—the site of action potential initiation. Since both ankyrins and L1CAMs contain multiple members localized to distinct subcellular compartments, we hypothesize that L1CAMs may function as subcellular compartmental labels, which direct GABAergic synapse targeting (Fig. 1). Third, we discovered that, following the initial targeting GABAergic axon to a defined subcellular domain, there is a prolonged period of maturation, characterized by elaboration of multiple axon terminals and proliferation of synapses. This maturation process in the primary visual cortex is potently regulated by neuronal activity and sensory experience during a postnatal critical period (Fig. 2), which may have profound impact on neuronal receptive field properties and the construction of functional cortical circuits. Fourth, we continue to make progress in studying the role of GABAergic inhibition in decoding the temporal pattern of neuronal input, and in regulating activity-dependent synaptic competition in the visual cortex.

Experience-independent Subcellular Synapse Targeting in the Primary Visual Cortex

G. diCristo, C. Wu, Z.J. Huang

The subcellular locations of synaptic contacts significantly impact their contributions to the integration and firing of postsynaptic neurons. Subcellular synapse targeting is especially prominent among different classes of GABAergic interneurons, which direct their synapses to restricted compartments of principal neu-

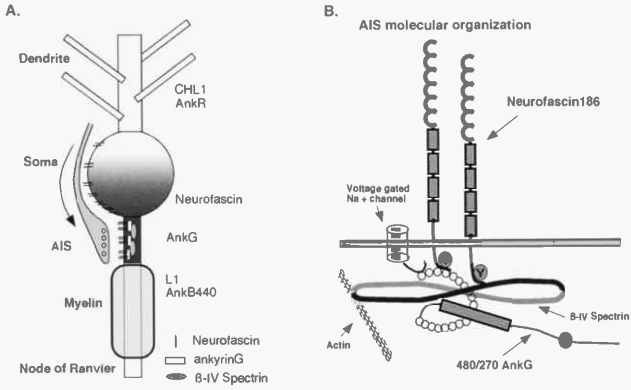

FIGURE 1 (*A*) A hypothetical model of subcellular molecular labels on a principal neuron which contribute to subcellular targeting of GABAergic synapses. The L1 family immunoglobulin cell adhesion molecules and ankyrin family membrane skeleton are differentially localized to subcellular compartments. A subcellular gradient of neurofascin directs basket synapses to the axon initial segment (AIS). (*B*) Molecular organization at AIS. Neurofasci 186 is recruited to AIS by binding to the ankyrin G and β-IV Spectin complex.

rons. Such spatial organization of GABAergic innervation is crucial for the temporal precision in their regulation of neuronal excitability, integration, and synchrony. The mechanism of subcellular synapse targeting is not well-defined, but it has been shown, in some species, to involve experience-driven synapse pruning. By combining cell-type-specific promoters, BAC (bacterial artificial chromosome) engineering, and two-photon imaging, distinct classes of GABAergic synapses and their postsynaptic pyramidal neurons were visualized in the primary visual cortex and in organotypic cultures of transgenic mice. We show that the subcellular organization of perisomatic and dendritic-targeted GABAergic innervations in organotypic cultures was indistinguishable compared to that in the primary visual cortex. Therefore, subcel-

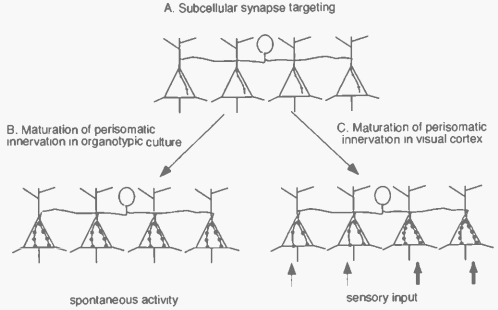

FIGURE 2 Experience-dependent maturation of perisomatic GABNAergic innervation in primary visual cortex. (*Triangles*) Pyramidal cells; (*circles*) basket interneurons; (*black dots*) perisomatic synapses. After initial targeting of basket axons to pyramidal cell soma, the formation of high.y distinct perisomatic synapses proceeds in organotypical culture and is regulated by neuronal spiking. In visual cortex, the maturation and pattern of perisomatic innervation may reflect differential activation of pyramidal neurons by visual input (represented by the thickness of upward arrows).

lular targeting of GABAergic synapses in the primary visual cortex is likely guided by cell surface molecular labels and experience-independent forms of neuronal activity.

Role of the L1 Family Immunoglobulin CAM Neurofascin in Synapse Targeting to the Axon Initial Segment

F. Ango, H. Yang, Z.J. Huang

Subcellular synapse targeting is most striking at the axon initial segments (AISs) of large projection neurons such as pyramidal and Purkinje neurons. Although only one micron in diameter and tens of microns in length, AIS is both the site of initiation of regenerative sodium spikes and the target of strong GABAergic inhibition. The degree of the spatial precision for this class of synaptic connection is striking as well as puzzling: How do GABAergic axons reach and recognize such a minute piece of cell membrane among all other possible targets in a highly heterogeneous tissue? Our study suggests that synapses targeting to AIS is achieved through a series of developmental events, each sequentially and cumulatively contributing to the final exquisite precision.

Using BAC transgenic mice that label basket interneurons during cerebellar circuit formation, we found that basket axons first contacted Purkinje soma before innervating AIS and forming the "pinceau synapses." Furthermore, we discovered a subcellular gradient of neurofascin, an L1 family immunoglobulin CAM, along the Purkinje AIS-soma membrane during the process of basket cell synapse targeting. The neurofascin gradient is dependent on ankyrin G, a membrane adapter protein exclusively localized to Purkinje AIS. In the absence of neurofascin gradient, basket axons lost directionality in their growth along Purkinje cells, were mistargeted, and precisely followed neurofascin distribution to ectopic locactions. Furthermore, formation of "pinceau synapses" was severely reduced along mistargeted basket axons. These results implicate an ankyrin-based subcellular gradient of neurofascin as a molecular determinant in directing axon targeting and GABAergic innervation at Purkinje AIS by cerebellar interneurons (Fig. 1). To provide further support for this conclusion, we are developing in vivo electroporation and viral infection procedures to directly manipulate neurofascin in the cerebellum.

Subcellular Molecular Labels and Their Receptors Underlying Synapse Targeting

F. Ango, H. Yang, Z.J. Huang

Our discoveries have raised many more questions than answers regarding the molecular mechanisms of subcellular synapse targeting. For example: Is the L1CAM neurofascin 186 involved in GABAergic innervation of neocortical pyramidal neurons, in addition to cerebellar Purkinje neurons, and therefore represents a general mechanism for synapse targeting to AIS? What is the mechanism for GABAergic synapse targeting to the dendritic compartment of principal neurons? What are the membrane receptors in GABAergic axon terminals, which interact with and "recognize" L1CAMs on principal neurons? We have evidence that both ankyrins and L1CAMs are also expressed in the developing and mature neocortex and hippocampus and are localized to axon initial segments of pyramidal neurons. We are testing the notion that the ankyrin-G-based neurofascin subcellular localization contributes to GABAergic synapse targeting in the neocortex, and thus represent a general mechanism for GABAergic synapse targeting to axon initial segment of principal neurons in the mammalian central nervous system (CNS). In addition, we are testing the hypothesis that another L1CAM, Close Homologue of L1 (CHL1), is involved in directing specific classes of GABAergic synapses to the dendritic compartment of principal neurons. Finally, we have initiated an effort to identify receptors for L1CAMs in GABAergic axon terminals by testing candidate genes and by characterizing gene expression profiles, especially that of CAMs, in defined classes of GABAergic interneurons.

Role of Glia Cells and Fyn Kinase Signaling in Subcellular Synapse Targeting

F. Ango, Z.J. Huang

At the pinceau synapses in the cerebellum, basket axon terminals at Purkinje AIS are closely surrounded by Bergmann glia processes and opposed by oligodendrocytes (myelin). We have found that defects in bas-

ket cell synapse targeting are closely associated with defects in the development and organization of glia processes. We are studying the role of glia cells in the subcellular targeting and maturation of pinceau synapses. In Fyn-deficient mice, there are both a delayed myelination and a persistent atrophy of Bergmann glia processes. The delayed myelination is correlated with an ectopic localization of neurofascin along Purkinje axons and ectopic targeting of basket cell axons. The atrophy of Bergmann glia processes is correlated with significantly reduced complexity of basket axon terminals. We are testing whether and how Fyn function is required in basket interneuron, Purkinje cells, or glia cells for the development of pinceau synapses.

Activity and Experience-dependent Maturation of GABAergic Innervation in Primary Visual Cortex during a Postnatal Critical Period

B. Chattopadhyaya, G. diCristo, Z.J. Huang [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory; G. Knott, University of Lausanne, Switzerland]

In the primary visual cortex, a single basket interneuron innervates hundreds of pyramidal neurons, and each pyramidal neuron receives inputs from multiple basket cells. At each postsynaptic target, a basket cell axon extends multiple terminals with large boutons clustered around pyramidal cell soma and proximal dendrites, forming the characteristic perisomatic synapses. Using BAC transgenic mice that specifically label perisomatic synapses from basket interneurons, we have characterized the development of perisomatic innervation in the primary visual cortex and studied the role of visual experience in the maturation of perisomatic innervation. We show that, following subcellular synapse targeting and initial synaptic contacts, there is a prolonged period of maturation of perisomatic innervation, characterized by elaboration of multiple axon terminals and proliferation of synapses among pyramidal neurons. Interestingly, perisomatic synapses formed in cortical organotypic cultures, through a stereotyped process involving the extension of distinct terminal branches and proliferation of perisomatic boutons.

Neuronal spiking was necessary for the proliferation of boutons and the extension but not the maintenance of terminal branches, thereby influencing the pattern of perisomatic innervation among pyramidal neurons. In the primary visual cortex, perisomatic innervation matured during a protracted period after eye opening and was significantly reduced by visual deprivation during the third but not the fifth postnatal week. Therefore, our data show that perisomatic synapse formation is driven by mechanisms intrinsic to cortical circuits, whereas the maturation and pattern of perisomatic innervation in the visual cortex are regulated by neuronal activity and sensory experience during a postnatal critical period (Fig. 2).

Molecular Mechanisms Underlying the Maturation and Plasticity of GABAergic Synapses in Neocortex

G. diCristo, B. Chattopadhyaya, C. Wu, Z.J. Huang

Although the mechanisms of activity-dependent plasticity at the glutamatergic synapses have been extensively studied and increasingly well understood, little is known about the mechanisms of morphological plasticity at the GABAergic synapses. The recapitulation of perisomatic GABAergic innervation in our organotypic culture systems establishes an efficient preparation to dissect the cellular and molecular mechanisms underlying the maturation and structural plasticity of GABAergic synapses. We have started to chronically image the dynamics and developmental process of somatic innervation using 2-photon microscopy. We are testing whether and how GABAergic axonal and synaptic morphogenesis can be induced and modified by electrical stimulation. Furthermore, using dominant-negative constructs and single-cell gene knockout, we are testing the role of GABAergic synaptic transmission, bone-derived neurotrophic factor (BDNF)/*trkB* signaling, and CAMs in the maturation and plasticity of GABAergic synapses.

GABAergic Mechanism of Critical Period Plasticity in Visual Cortex

S. Kuhlman, Z.J. Huang

The cellular mechanisms by which GABAergic circuits regulate ocular dominance plasticity and the critical period are unknown. We hypothesize that during

the critical period, maturation of synaptic inhibition allows cortical circuits to detect the difference between the temporal patterns of open and closed eye inputs, and engage spike-timing-dependent plasticity rules to drive synaptic competition and ocular dominance (OD) plasticity.

We have made the following observations using in vitro visual cortical slice preparations which support our hypothesis: First, we demonstrated that the strength of synaptic inhibition converging onto layer-2/3 pyramidal cells increases with the progression of the critical period. Second, we developed a two-pathway stimulation paradigm to demonstrate that suprathreshold input from one pathway (mimicking the open-eye input) preceding subsequent input from a second, independent pathway (mimicking closed-eye input) actively suppresses the second pathway from driving postsynaptic spikes. This effect of spike suppression does not occur at early postnatal ages, and the onset of suppression correlates with the maturation of GABAergic transmission and peak of the critical period. Third, initial experiments suggest that repeated trials of input-specific spike suppression leads to a persistent decrease in spike probability, consistent with the idea that a spike-timing-dependent plasticity mechanism induces long-term depression (LTD) at synapses, which are actively excluded from contributing to postsynaptic spike generation. The time interval of spike suppression between the stimulation of the two pathways (~40–60 ms) suggests that the recruitment of heterosynaptic inhibition by the preceding pathway may mediate suppression. To test this notion, we are developing tools to specifically reduce synaptic inhibition in single pyramidal cells by disrupting the GABA-A receptor $\gamma 2$ subunit, a critical component required for the clustering of functional GABA receptors at synapses. We are using a strain of conditional knockout mice in which the $\gamma 2$ subunit is flanked by *loxP* sites. Preliminary results suggest that adeno-associated virus-mediated expression of Cre recombinase in combination with green fluorescent protein (GFP) in layer-2/3 cortical neurons in vivo may be an effective method to delete genes in neurons and to mark these neurons for electrophysiologic recording in a background of wild-type neurons. This strategy thus allows the role of synaptic inhibition to be specifically studied within an otherwise normally functioning network. We expect spike suppression to be absent in neurons with disrupted $\gamma 2$.

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THEORETICAL NEUROBIOLOGY AND QUANTITATIVE PRINCIPLES OF CORTICAL DESIGN

A. Koulakov D. Tsigankov

This year we concentrated on the role of the brain as a "statistical" computational device. The premise is that in many instances the nervous system acts as a Brownian ratchet, which rectifies stochastic behavior of its components to yield deterministic responses. In this general direction, we have made two advances this year. First, we understood how to identify components causally linked to behavior in statistical computational devices, such as brain networks. The definitions we have designed are not quite useful to be directly applied experimentally. Yet, we have cleared the ground for more links to experiment with next year. Second, we created a mathematical model for development of sensory maps in the brain, which involves random search directed by molecular cues. This model can account for several intriguing experimental findings. Our next step is to understand how neuronal activity contributes to refinement of neuronal connectivity and competition in a way consistent with existing experimental data.

Decision-making Analysis

D. Tsygankov, A. Koulakov [in collaboration with D. Rinberg, Monell Chemical Senses Center, Philadelphia, Pennsylvania]

The nervous system often faces the problem of classifying stimuli into discrete groups and responding on the basis of these classifications. Neurons involved in these tasks can be separated into three groups: sensory elements, whose activity is highly correlated with the stimulus; decision-making (DM) elements, which are responsible for classifying the stimuli; and motor elements, which implement the results of such classifications. Although sensory and motor groups can be identified experimentally, e.g., by studying correlations with stimulus and response, there is no acceptable definition for DM elements. In this project, we develop a mathematical definition of DM components and design experimental strategies, which allow determination of DM elements from anatomical and physiological data. Such decision-making analysis (DMA) has practical importance, since once units involved in making a particular decision are located, further efforts can be concentrated on uncovering the underlying neuronal mechanisms.

Localization of DM elements and, more generally, localization of brain function have a long history. Perhaps, the first important step was the transition from cardiocentric to encephalocentric views on behavior. Thus, ancient Egyptians thought that the heart was the most important organ in the human body, the seat of the mind, the center of intellectual activities. To emphasize this, in the process of mummification, they carefully embalmed the heart, liver, and other organs, whereas the brain was scooped out through the nostrils. By the second-century A.D., Greeks realized that the nervous system must include two fundamental components: sensory and motor limbs. DM occurs on the interface between them and is accomplished by an intermediate nonphysical process called the soul. The argument was raging about the location of the point between the sensory and motor limbs, which is the seat of the soul. Some, following Aristotle, defended the cadiocentric system, while others agreed with Plato and Hippocrates that the soul is located within the ventricles of the brain. Only in the second-century A.D. did the Greek physiologist Galen conduct experiments showing the critical importance of the brain for behavior. However, it was not until the age of Enlightenment that the encephalocentric views were universally accepted.

The next important step was the distinction between two types of DM elements, made by René Descartes. He suggested that some responses could be explained materialistically by the processes in networks of fibers passing through the brain. The decisions in this case are deterministic and amenable to physiological study. In other cases, decisions are not deterministic and involve intervention of a nonmaterial soul, in which case, they could be considered voluntary. The distinction between deterministic and sto-

chastic behaviors forms the foundation for the current understanding of DM.

Recently, we initiated a theoretical study of localization of decision makers in neural networks. The goal of this study is to discover a mathematical definition of DM. Such a definition would allow us to measure and locate the network components responsible for a given function, which would move the dispute between holistic and localized views on brain function to the domain of empirical science. At the heart of our definition is the stochastic component of behavior, which was emphasized by Descartes. As the main tool, we assume the use of observational noninvasive techniques. Therefore, we aim our studies at the human nervous system, where anatomical, MRI, MEG, and other techniques could be used. At the same time, since network architecture is universal in biology, our analysis could be applied to molecular, neuronal, and social networks.

Models for Molecular Development of Sensory Maps

D. Tsygankov, A. Koulakov

We examined results of gain-of-function experiments on retinocollicular maps in knockin mice (Brown et al., *Cell 102:* 77 [2000]). In wild-type mice the temporal-nasal axis of the retina is mapped to the rostral-caudal axis of the superior colliculus (SC). The established map is single-valued, which implies that each point on the retina maps to a single termination zone (TZ) in the SC. In homozygous Isl2/EphA3 knockin mice, the map is double-valued, which implies that a single point on the retina maps to two TZs in the SC (Fig. 1). This is a reflection of the fact that only about 50% of the cells in the retina express Isl2. In heterozygous Isl2/EphA3 knockins, the map is intermediate between the homozygous and wild type: It is single-valued in temporal and double-valued in the nasal parts of retina.

We study map formation using a stochastic model based on Markov chains. In our model, the map undergoes a series of reconstructions with probabilities dependent on a set of chemical cues. Our model suggests that the map in heterozygotes is single-valued in the temporal region of the retina due to a reduced gradient of ephrin-A in the corresponding region of the SC. The remaining map is double-valued since the gradient of ephrin-A is high there. We therefore predict that if the gradient of ephrin-A is reduced by a genetic manipulation, the single-valued region of the map should occupy a larger portion of the temporal retina; i.e., the point of transition between single- and double-valued maps should move to a more nasal position in Isl2-EphA3 heterozygotes. We also discuss the importance of the inhomogeneous EphA gradient.

We therefore present a theoretical model for retinocollicular map development, which can account for intriguing behaviors observed in gain-of-function

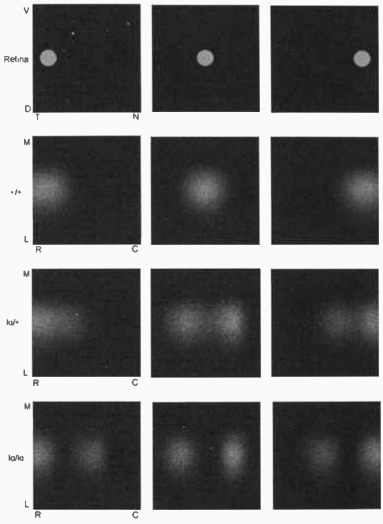

FIGURE 1 Model for retinocollicular map development. The top row shows anterograde "labeling spots" in the retina. The following three rows display the corresponding distribution of labels in the superior colliculus. The size of both retinal and collicular arrays is 100 x 100 cells. The three rows show results for wild-type, heterozygous knockins, and homozygous Isl2/EphA3 knockins, as marked on the left. Notice the doubling transition, when going from temporal to nasal injection in heterozygotes. (D) Dorsal; (V) ventral; (N) nasal; (T) temporal; (C) caudal; (R) rostral; (L) lateral; (M) medial.

experiments by Brown et al. (*Cell 102:* 77 [2002]) including map bifurcation in heterozygous Isl2/EphA3 knockins. The model is based on known chemical labels, axonal repulsion/competition, and stochasticity. Mapping in Isl2/EphB knockins is also addressed.

Repulsion Induced by Attraction: A Role of Ephrin-B1 in Retinotectal Mapping

D. Tsygankov, A. Koulakov

We study the role of EphB receptors and their ligand ephrin-B1 in dorsalventral retinotectal mapping. It was suggested earlier that ephrin-B1 may act as an attractant of EphB+ axons of retinal ganglion cells. We addressed the results of a more recent experiment with chicks (McLaughlin et al., *Development 130:* 2407 [2003]) in which axons of retinal ganglion cells were shown to be repelled by high ephrin-B1 density. It was therefore proposed that ephrin-B1 might act as both attractant and repellent. We showed that the same axonal behavior may follow from attraction to ephrin-B1 density and axonal competition for space. Therefore, we showed how apparent repulsive interaction can be induced by a combination of attraction to the target and competitive interactions between axons. We suggested experimental tests that may distinguish repulsive interaction with the target from repulsion induced by attraction and competition.

Combinatorial Model for Olfactory Coding

D. Tsygankov, A. Koulakov [in collaboration with D. Rinberg, Monell Chemical Senses Center, Philadelphia, Pennsylvania]

We analyzed a model for olfactory coding based on spatial representation of glomerular responses. In this model, distinct odorants activate specific subsets of glomeruli, dependent on the odorant's concentration. The glomerular response specificities are understood statistically, based on experimentally measured distributions of odor detection thresholds. A simple version of the model, in which glomerular responses are binary (the on-off model), allows us to quantitatively account for the following results of human/rodent psychophysics: (1) just noticeable differences in perceived concentration of a single odor (Weber ratios) are $dC/C \sim 0.1$; (2) the number of simultaneously perceived odors can be as high as 12 (Jinks and Laing, *Perception 28:* 395 [1999]); (3) extensive lesions of the olfactory bulb do not lead to significant changes in detection or discrimination thresholds. A more detailed model allows us to reproduce closely the conditional probabilities obtained in human psychophysical experiments on perception of complex odors.

PUBLICATIONS

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THE NEURAL ORGANIZATION OF OLFACTORY BEHAVIOR

Z.F. Mainen V. Egger, C. Feierstein, E. Friedman, H. Gurden, A. Kepecs, M. Quirk, S. Ranade, D. Sosulski, N. Uchida, H. Zariwala

Our laboratory is interested in the neural basis of mammalian behavior. We focus on understanding the nature of the electrical activity of single neurons, the chief currency through which genes and molecules express themselves in the functioning brain. We are using the rodent olfactory system as a model for studying neural coding, the problem of how information is represented in neuronal spike trains. We view neural coding in the broader framework of decision-making, i.e., how neurons work together to select one of a repertoire of possible motor actions on the basis of experience, current goals, and sensory data. We are especially interested in the temporal coordination of activity between neurons and between regions of the brain that is necessary for coherent and directed action. Our principal experimental technique is chronic multi-electrode recordings in rodents trained to perform sensory discrimination tasks. We are actively developing new ways of monitoring and controlling both behavior and neural activity. We are also using intrinsic optical imaging to study the link between electrical activity (information) and metabolic activity (energy) in the brain and 2-photon imaging and whole-cell recording to study the synaptic and electrical properties of individual neurons.

To help understand the relationship between neural phenomena and behavioral phenomena, we are using neural network and statistical theories that can be expressed in computational models and thereby related to our data. Our long-term goal is to apply these approaches and insights toward human psychiatric disease, especially schizophrenia.

Psychophysical Constraints on Olfactory Coding

N. Uchida

How is odor information coded in the brain? Psychophysical experiments can have an important role in elucidating neural coding because behavior is the ultimate readout of the neural code. Reaction time experiments, in which the time it takes to make a decision is measured, can provide particularly crucial constraints on the temporal aspects of neural coding. Olfaction is sometimes thought of as a slow sense, and thus it has been proposed that relatively slow temporal processing might have a role in odor coding. We recently tested this idea using an odor mixture discrimination task in rats (Fig. 1). We found that across a wide range of conditions, rats discriminated odors very rapidly, with a median odor sampling duration of <300 msec (Uchida and Mainen 2003). More surpris-

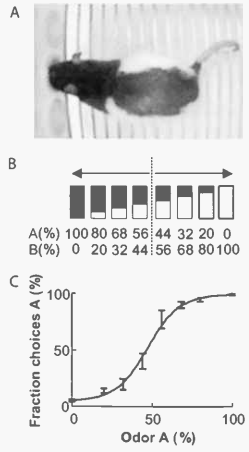

FIGURE 1 Two-alternative-odor mixture discrimination task. (*A*) A rat performing odor discrimination task. Rats can perform this task with ~200 ms of odor sampling regardless of discrimination difficulties. (*B*) Mixture stimuli. Two odors were mixed with various proportions, and rats were trained to report the dominant component by making left or right choice. (*C*) Typical performance in odor mixture discrimination task.

ingly, measurement of the rats' sniffing revealed that optimal performance was achieved with only one sniff at theta frequency (~7 Hz). These results tell us that the neural code for odors must evolve very quickly, on the time scale of tens of milliseconds. Such a rapid process might utilize synchrony or phase information for coding. The results also suggest to us that the sniffing (theta) cycle may be important in coordinating the neural activity during olfactory perceptual decisions in the rat. We have been investigating these ideas by recording electrical activity using chronic recordings from behaving rats.

Concentration Invariant Odor Quality Discrimination

N. Uchida

Any odor, whether a single chemical or a complex mixture, activates a specific combination of odorant receptors. It has therefore been postulated that combinations of activated receptors can define odor quality ("combinatorial coding"). However, changes in concentration produced by changes in distance from an odor source will also generally lead to changes in the identities of activated receptors, as higher concentrations will recruit new lower-affinity receptors. It would be useful for a creature to be able to recognize these different combinations as belonging to the same source. But do animals in fact perceive odor quality in a concentration-invariant fashion? Although anecdotal evidence exists, this issue has not been investigated quantitatively.

Using the two-alternative odor mixture discrimination task (Fig. 1), we tested explicitly the idea that a rat perceives mixture qualities to be invariant to the ratios of their components. After training, rats were tested using probe stimuli with identical ratios as the training set but at different absolute concentrations. Performance was predicted by a ratio-based discrimination strategy but not by alternatives such as an intensity-based discrimination strategy. This result indicates that molar ratios of the components of a mixture are the critical determinants of choice in this task. We propose that odor quality is coded by the ratios of ligand coverage of odorant receptors, "ratio coding." To understand how ratio coding might arise from ensemble activity in the olfactory bulb and cortex, we are currently recording spike activity in behaving rats using multi-electrode techniques.

Effects of Sensory and Reward Experience on Olfactory Decisions

H. Zariwala, N. Uchida, A. Kepecs

One might think of the job of the rat in our task as determining the identity of the odors it is presented, but behavioral ecology suggests that the rat is really just interested in getting as much water as fast as it can, i.e., animals make foraging decisions so as to maximize reward and minimize work. Therefore, to understand the decisions that rats make in this task, sensory information must be considered in the context of the incentives afforded by the task. For example, if more water is being delivered from the right choice port than the left, this will bias a rat's choices to the right when the stimuli are ambiguous. With the eventual goal of elucidating the underlying neural mechanisms, we are investigating how olfactory decisions are affected by the animal's experience with stimuli and rewards.

For example, in a recent study, we examined the effect of changing the stimulus context on a rat's performance. We found that rats substantially improved their performance when a single difficult stimulus pair was presented repeatedly in a session compared to the standard condition in which the whole range of easy and difficult mixtures is interleaved. This effect reversed immediately when stimulus context was switched back, indicating a rapid and transient underlying plasticity. Interestingly, the performance gain was achieved without an increase in response time—there was no speed-accuracy tradeoff. We are currently designing experiments to tease apart the possible roles of feed-forward sensory adaptation from reward-dependent learning in this phenomenon.

Single-neural Correlates of Olfactory Behavior in Olfactory and Prefrontal Cortex

C. Feierstein, M. Quirk

To investigate the neural substrates of olfactory-guided decisions, we are using chronic multi-electrode recording techniques to monitor the activity of ensembles of neurons in rats while they perform the two-alternative-odor discrimination task (Fig. 1). Our studies have focused on the primary olfactory or piriform cortex and the orbitofrontal cortex, a secondary olfactory area that is part of the rodent prefrontal cortex.

Neurons in both orbitofrontal and piriform cortex display a wide range of different behavioral correlates. In both areas, some neurons respond in an odor-selective fashion during the delivery of the stimulus, whereas many other respond selectively to other aspects of the task such as the delivery of water reward. In this way, both of these areas appear to be well-suited to a role in linking the different kinds of information necessary to learn and execute olfactory perceptual decisions. What then distinguishes their respective roles in these processes?

Our preliminary results provide a number of clues to this question. These recordings show that during the course of the odor-sampling period, odor-selectivity develops more rapidly in the piriform cortex than in the orbitofrontal cortex. On the other hand, there is a richer representation of reward information in orbitofrontal cortex. Both areas contain a large portion of neurons that fire selectively during the response period. These observations are consistent with a scenario in which the piriform cortex is specialized for rapid responding, whereas the orbitofrontal cortex is specialized for rapid learning.

Local Competitive Interactions in Rat Prefrontal Microcircuits during Binary Olfactory Decisions

M. Quirk, C. Feierstein

Although much is known about the cellular and synaptic components of cortical circuits, considerably less is known about how these circuits behave in the functioning brain. Using multi-electrode recordings, we are able to isolate action potentials arising from multiple cells within a small local volume of tissue (diameter <100 μm) corresponding to a cortical microcolumn. By applying these recordings to the prefrontal cortex of rats performing our two-alternative olfactory discrimination task (Fig. 1), we are studying how behaviorally relevant information is represented within cortical microcircuits during the formation of a binary perceptual decision. In contrast to the typical columnar organization of primary sensory areas, even nearby cells display diverse response properties. The heterogeneity of responses across cells recorded in the same electrode was comparable to that of cells across electrodes, indicating that the salient task variables represented in prefrontal cortex are available locally within individual microcolumns. Analysis of the interactions between simultaneously recorded neurons shows that nearby cells are often anticorrelated, suggesting competition between neighboring neurons whereby functionally distinct subsets of cells actively suppress the firing of other local neurons. Such competitive interactions within prefrontal cortical microcircuits suggest a mechanism for the creation of diverse local responses and provide a potential substrate for "winner-take-all" computations that may be essential components of neural computation of decisions.

Role of the Theta Cycle in Coordinating Brain Regions during Perceptual Decisions

A. Kepecs

Rhythmic oscillations in the theta frequency band (4–10 Hz) are observed in local field potentials recorded from many different cortical and subcortical brain regions in rodents. The presence and amplitude of theta oscillations is correlated with specific behavioral states, being particularly prominent during active, exploratory behaviors. In addition, the phase of theta oscillations can be reset by behaviorally relevant stimuli. Theta oscillations may have different functional roles in different brain regions and behavioral contexts, but anatomical and physiological evidence suggests that theta oscillations can coordinate information flow between brain regions underlying sensorimotor integration. To elucidate the neural mechanisms behind this coordination, we are recording local field potentials (LFPs) simultaneously in the olfactory bulb, olfactory cortex, and hippocampus in rats performing an olfactory discrimination task (Fig. 1). We observe both theta (4–10 Hz) and higher-frequency (beta: 15–25 Hz and gamma: 30–50 Hz) oscillations at several recording sites. Currently, we are analyzing how different spectral features of LFPs correspond to different behavioral epochs and the nature of the relationship between oscillations in different brain regions.

Neural and Metabolic Activity in Olfactory Glomeruli

H. Gurden, S. Ranade, N. Uchida

Neural activity is mirrored by metabolic activity which in turn causes physiological signals that can be detected using functional imaging techniques. The mechanisms that link these processes are the subject of intense interest. We are investigating the link between odor-evoked neural activity and intrinsic optical sig-

nals (IOSs) in the olfactory glomeruli. IOSs are used for studying the representation of odors, but we hope to clarify how to interpret IOS as a readout of glomerular activity and the mechanisms that link neural activity to metabolism and functional imaging signals.

We measure odor-evoked IOSs bilaterally in the dorsal olfactory bulb of the acutely anesthetized rat. Odor stimulation evokes three types of changes in the reflectance of red light. The first two components are a punctate and a diffuse transient decrease in reflection whose spatial localization is odor-specific. The third component is a complex, oscillatory signal (~0.1 Hz) that is present in the absence of stimulation. To examine the cellular mechanisms that link neural activity to IOS, drugs are applied to one bulb and another bulb serves as a control. Odor-evoked IOS are blocked by the Na^+ channel blocker TTX, confirming that they are triggered by neuronal activity. IOSs are reduced by dopamine and GABA-B agonists but are not blocked by glutamate receptor antagonists (NBQX+APV). Taken together, our data indicate that IOS in the olfactory glomeruli depends on presynaptic transmitter release but not on transmission through postsynaptic excitatory synapses. The results also indicate that periglomerular cells could control the gain of receptor neuron input to the olfactory bulb.

We are currently working to adopt these imaging methods to chronic recording in the awake rat. In this preparation, we will examine the impact of active sampling (sniffing) and begin to dissect how brain states (sleep, wakefulness), attention, and learning modulate odor representations and their coupling to metabolic activity.

Calcium Dynamics Underlying Dendritic Integration in Olfactory Bulb Granule Cells

V. Egger [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory]

In the mammalian olfactory bulb, granule cells (GCs) mediate lateral inhibition, the main form of synaptic processing in the olfactory bulb. Although GCs can fire action potentials (APs), they lack an axon and may therefore rely on various forms of dendritic signaling to couple their synaptic input to the release of inhibitory neurotransmitter. To better understand signal integration in these cells, we have been using 2-photon microscopy in rat brain slices to monitor calcium transients in GC apical dendrites and spines. We find that APs produce robust calcium influx uniformly throughout the dendritic tree and spines of GCs and hence contribute to a kind of global lateral inhibition. Interestingly, these global signals are carried in large part by T-type calcium channels (Egger et al. 2003). When GCs are activated synaptically (through extracellular stimulation in the glomerular layer), global calcium signals can also be produced in the absence of APs. This global subthreshold signal is entirely T-channel-mediated and appears to reflect a low-threshold calcium spike. It represents a novel pathway for AP-independent lateral inhibition in the olfactory bulb. Finally, at the level of individual responsive synapses, robust calcium transients are strictly localized to the spine and do not invade the parent dendrite. These signals are carried by a mixture of NMDA (*N*-methyl-D-aspartate) receptors and voltage-dependent calcium channels. In addition, they are substantially boosted by calcium-induced calcium release from thapsigargin-sensitive internal stores. This local synaptic activity can interact with the global signals evoked by APs or synaptic events, sometimes producing supralinear calcium transient amplitudes.

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TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow A. Barria, C. Becamel, J. Boehm, N. Dawkins, I. Ehrlich, H. Hsieh, F. Kamenetz, C. Kopec, B. Li, A. Piccini, K. Seidenman, T. Takahashi

This laboratory is directed toward an understanding of learning and memory by studying the physiology of synapses. We study synaptic transmission and plasticity in rodent brains, and use both in vitro brain slices and in vivo brains. The brain slice preparation is sufficiently complex to show glimpses of emergent properties, as well as simple enough to allow hard-nosed biophysical scrutiny. We apply the knowledge and reagents gained from these studies to test models of neural plasticity in brain/behavior paradigms. To monitor and perturb the function of synapses, we use a combination of electrophysiology, microscopic imaging and molecular transfection/infection techniques, which allows us to examine the cellular and molecular basis for changes in electrophysiological function. Last year, we added a new preparation, in which we acutely deliver genes of interest into living animals with viruses and examine the impact of experience on the subcellular distribution of these gene products. We are now testing the role of specific forms of synaptic plasticity in various brain regions during behavioral paradigms. Such experiments provide support to our philosophy that synapses have key properties whose understanding will provide insight into phenomena at higher levels of complexity. Below are discussed examples of projects recently published and their subsequent progress.

PKA PHOSPHORYLATION OF AMPA-R SUBUNITS CONTROLS SYNAPTIC TRAFFICKING UNDERLYING PLASTICITY

The regulated incorporation of α-amino-3-hydroxy-5-methyl-4-isoazole (AMPA) receptors (AMPA-Rs) into synapses is important for synaptic plasticity. Here, we examine the role of protein kinase A (PKA) in this process. We found that PKA phosphorylation of the AMPA receptor subunits GluR4 and GluR1 directly controlled the synaptic incorporation of AMPA receptors in organotypic slices from rat hippocampus. Activity-driven PKA phosphorylation of GluR4 was necessary and sufficient to relieve a retention interaction and drive receptors into synapses. In contrast, PKA phosphorylation of GluR1 and the activity of calcium/calmodulin-dependent kinase II (CaMKII) were both necessary for receptor incorporation. Thus, PKA phosphorylation of AMPA-R subunits contributes to diverse mechanisms underlying synaptic plasticity.

We are currently identifying additional phosphorylation sites on AMPA-Rs that control plasticity. Our current view is that multiple phosphorylation steps are involved in various aspects of receptor trafficking, such as relief from extrasynaptic retention, integration into the dendritic surface, access to the perisynaptic region, integration into the synapse, anchoring to the synapse, and removal from the synapse. For instance, by mimicking phosphorylation at four sites on the AMPA-R cytoplasmic tail, we can drive receptors into synapses. We are also taking a proteomic approach to determine what protein-protein interactions are enhanced/removed by phosphorylation events.

GLUTAMATERGIC PLASTICITY BY SYNAPTIC DELIVERY OF GLUR-2(LONG)-CONTAINING AMPA-Rs

Activity-driven delivery of AMPA-Rs is proposed to mediate glutamatergic synaptic plasticity, during both development and learning. In hippocampal CA1 principal neurons, such trafficking is primarily mediated by the abundant GluR-1 subunit. We now report a study of GluR-2(long), a carboxy-terminal splice variant of the GluR-2 subunit. GluR-2(long) synaptic delivery is regulated by two forms of activity. Spontaneous synaptic activity-driven GluR-2(long) transport maintains one third of the steady-state AMPA-R-mediated responses, whereas GluR-2(long) delivery following the induction of LTP is responsible for approximately 50% of the resulting potentiation at the hippocampal CA3 to CA1 synapses at the time of GluR-2(long) peak expression, the second postnatal week. Trafficking of GluR-2(long)-containing receptors thus mediates a GluR-1-independent form of glu-

tamatergic synaptic plasticity in the juvenile hippocampus.

CONTROL OF NMDA-R TRAFFICKING TO SYNAPSES BY THE NR2 SUBUNIT

To elucidate mechanisms controlling the number and subunit composition of synaptic *N*-methyl-D-aspartate receptors (NMDA-Rs) in hippocampal slice neurons, the NR1, NR2A, and NR2B subunits were optically and electrophysiologically tagged. The NR2 subunit directs delivery of receptors to synapses with different rules controlling NR2A and NR2B. NR2B-containing receptors incorporate into synapses in a manner that is not limited by synaptic transmission nor enhanced by increased subunit expression. NR2A-containing receptors, whose expression normally increases with age, replace synaptic NR2B-containing receptors. Replacement is enhanced by increased NR2A expression, requires synaptic activity, and leads to reduced NMDA-R responses. Surprisingly, spontaneously released transmitter acting on synaptic NMDA-Rs is sufficient for replacement. Thus, as with AMPA-Rs, synaptic trafficking of NMDA-Rs is tightly regulated and has unique rules.

We are currently testing the impact of the NR2B to NR2A switch on synaptic plasticity. Our preliminary results indicate that driving this switch reduces the generation of LTP. This reduction in plasticity is largely due to differences in affinity by NR2A and NR2B to CaMKII.

HOMEOSTATIC FEEDBACK BETWEEN NEURONAL ACTIVITY AND APP PROCESSING

A large body of work implicates the accumulation of Aβ's disease. However, little is known regarding the relation between neuronal electrophysiological function and APP processing. We find here that neuronal activity controls the formation of Aβ. Furthermore, increased formation of Aβ reversibly depresses synaptic function. These results suggest that APP cleavage products act as negative feedback homeostatic regulators that keep neuronal hyperactivity in check. Pathology that disrupts this homeostatic system could increase the production of Aβ and/or produce neuronal hyperactivity, events that may contribute to Alzheimer's disease.

We are currently examining the synaptic process affected by Aβ responsible for depressed transmission. Our preliminary evidence indicates that AMPA-R trafficking is affected.

EXPERIENCE STRENGTHENS TRANSMISSION BY DRIVING AMPA-Rs INTO SYNAPSES

To elucidate the mechanisms underlying experience-dependent plasticity in the brain, the trafficking of the AMPA subclass of glutamate receptors into synapses was examined in the developing rat barrel cortex. In vivo gene delivery was combined with in vitro recordings to show that experience drives recombinant GluR1, an AMPA-R subunit, into synapses formed between layer 4 and layer 2/3 neurons. Furthermore, expression of the GluR1 cytoplasmic tail, a construct that inhibits synaptic delivery of endogenous AMPA-Rs during long-term potentiation, blocked experience-driven synaptic potentiation. In general, synaptic incorporation of AMPA-Rs in vivo conforms to rules identified in vitro and contributes to plasticity driven by natural stimuli in the mammalian brain.

We are currently testing whether a similar process occurs during learning paradigms such as fear conditioning and cross-modal plasticity in adult animals.

PSD-95 AS A CRITICAL SYNAPTIC SCAFFOLD REQUIRED FOR LTP

The regulated delivery of AMPA-type glutamate receptors (AMPA-Rs) to synapses is an important mechanism underlying synaptic plasticity. Here, we ask whether the synaptic scaffolding protein PSD-95 (postsynaptic density 95) participates in AMPA-R incorporation during two forms of synaptic plasticity. In hippocampal slice cultures, the expression of PSD-95-green fluorescent protein (PSD-95-GFP) increases AMPA-R currents by selectively delivering glutamate receptor 1 (GluR1)-containing receptors to synapses, thus mimicking long-term potentiation (LTP). Mutational analysis shows that the amino-terminal of PSD-95 including the first two PDZ [PSD-95/Discs large (Dlg)/zona occludens-1 (ZO-1)] domains is necessary and sufficient to mediate this effect. Further supporting a role in synaptic plasticity, wild-type PSD-95 occludes LTP, and dominant-negative forms block LTP. Moreover, we demonstrate that PSD-95 also participates in AMPA-R delivery during experience-driven plasticity in vivo. In the barrel cortex from experience-deprived animals, the expression of PSD-95-GFP selectively increases AMPA-R currents,

mimicking experience-driven plasticity. In nondeprived animals, PSD-95-GFP produces no additional potentiation, indicating common mechanisms between PSD-95-mediated potentiation and experience-driven synaptic strengthening. A dominant-negative form of PSD-95 blocks experience-driven potentiation of synapses. Pharmacological analysis in slice cultures reveals that PSD-95 acts downstream from other signaling pathways involved in LTP. We conclude that PSD-95 controls activity-dependent AMPA-R incorporation at synapses via PDZ interactions not only during LTP in vitro, but also during experience-driven synaptic strengthening by natural stimuli in vivo.

Currently, we are employing RNAi (RNA interference) techniques with viruses to knock down PSD-95 levels in neurons and determine the effect on synaptic transmission and plasticity in in vitro and in vivo preparations.

In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission and plasticity and testing how these mechanisms participate in experience-driven plasticity.

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Helen Hsieh

COMPUTATIONAL AND SYSTEMS NEUROBIOLOGY

P. Mitra R. Baptista
H. Bokil
H. Maniar

This research effort began in the fall of 2003 when Partha Mitra joined the faculty at CSHL. Partha was previously located at Bell Laboratories, Lucent Technologies. Hemant Bokil, previously a postdoctoral fellow at Bell Laboratories working jointly with Partha and Richard Andersen at Caltech, also moved here to CSHL. Hemant continues his work on signal processing applications to neurobiological time series with special emphasis on neural prostheses. Rafael Baptista, a professional software developer who started out with some work on the Human Genome Project and has more recently been working in the video game industry, joined the research team in late August and will be performing the dual role of providing software infrastructure for the group and participating in the open source software project *Chronux* to develop software for neurobiological data analysis. Hiren Maniar also joined the group in August as a postdoctoral fellow, working as part of a consortium funded by the Human Frontiers Science Foundation to work on the role of neural synchrony in cross-modal integration.

Apart from this group of core personnel, our research effort includes close collaborative ties with multiple research groups in the area. A collaborative effort with Niko Schiff and Keith Purpura at the Weill Medical School of Cornell University, where Partha is an adjunct associate professor, involves electrophysiology in the awake behaving macaque, and members of the research team there include Alexander Polyakov, a postdoctoral member of the laboratory, and David Menzer, a graduate student at Weill Medical School. A second research effort on how zebra finches learn their song vocalizations is being carried out in collaboration with Ofer Tchernichovski at City College of New York. A third collaboration is with the group of Rodolfo Llinas and Urs Ribary at New York University Medical College, involving dynamic brain imaging using magnetoencephalography as the probe. A fourth consortium-based research effort to study cross-modal integration between the senses is funded by the Human Frontiers Science Program, including the laboratories of Shinsuke Shimojo (Caltech), Pascal Fries (F.C. Donders Research Center at Nijemegen), Shiny'a Nishida at the Nippon Telegraph and Telephone Corporation labs in Japan, and Alan Johnston at the University College, London.

Our basic research philosophy is to bring computational and theoretical tools to bear on biological questions in general, and neurobiological questions in particular, to make sense of biological complexity and to provide understanding and potential therapies for disease. There are two basic branches to the research: Neuroinformatics and Theoretical Engineering. The former involves the application of statistical and computational tools to neurobiological data, primarily single-channel and multichannel time series, including point processes (spike trains) and brain imaging data. The applications are geared toward exploratory analysis of large volumes of data, as well as confirmatory analysis for testing of specific hypotheses. This is a continuing research effort that reflects several years of work and is associated with a course entitled "Neuroinformatics" held each summer at the Marine Biological Laboratories in Massachusetts. The second branch of research deals with the question of theoretical principles underlying the design of biological systems. Since biological organisms are evolved to perform their tasks robustly under uncertain circumstances, in terms of both the environment and parametric uncertainty in components making up the system, it is expected that the mathematical theories developed by engineers to help design man-made systems that perform under similarly challenging circumstances may also apply to these systems. The goal of this research is to identify the engineering principles most germane to biology and to study selected examples drawn from different levels of the organizational hierarchy.

Chronux: Open Source Software for Analyzing Neurobiological Time Series Data

R. Baptista, H. Bokil

The nervous system stores and executes behaviors and adapts these behaviors to the environment through

sensory monitoring. Studies of the activity of the nervous system consist primarily of monitoring the dynamics of units at different levels of hierarchy of the system: ranging from single neurons measured electrically to large groups of neurons monitored indirectly through a variety of imaging methodologies. The relevant measurements consist of single or multichannel time series data, in the form of continuous or point processes. Therefore, the statistical analysis of time series data is central to studying the nervous system.

During the last several years, our research has involved the development of algorithmic and computational tools for the analysis of multichannel time series data from the neurosciences. We are now encoding these tools into an open source software package entitled *Chronux*. This project will proceed in stages and will involve the development of a high-quality numerical analysis library, data IO and management utilities, and a user interface that gives experimental neuroscientists access to advanced analysis tools. The project will proceed in stages, where we move from the current implementation of the relevant routines as MATLAB toolboxes to a fully open source and self-contained software. Although a number of signal processing libraries are available, the goal of this effort is to develop domain-specific tools that will aid neuroscience research. We expect that such tools will be critical to advancing our understanding of systems neuroscience.

Neural Prosthetics: Local Field Potential Measurements

H. Bokil

A number of current research efforts aim at developing invasive neural prosthetics, which will use electrical signals measured from microelectrodes implanted into appropriate brain regions to infer the motor or cognitive intentions of patients with peripheral neuropathology to drive a prosthetic device, or potential to stimulate a limb. Previously, these efforts have focused on spiking activity measured using microelectrodes. However, unit activity is not necessarily robust over periods of days, and gliosis at the electrodes may further degrade the measured signals. We have previously found that the local field potential (LFP) signal, i.e., low-frequency electrical recordings reflecting the extracellular currents flowing in the vicinity of the microelectrode tip, may be used as a robust alternate source of information about the underlying neural signals. In collaboration with Richard Andersen at Caltech, we have been working on utilizing these LFP signals for prosthetic applications. We have recently finished development of an event detection and classification framework for this purpose and have tested it on a set of recordings from the intraparietal sulcus in macaque performing a working memory task. We find that for the signals in this brain region (relating to saccadic eye movements), the LFPs are equally informative, and more robust, than the unit activity measured by individual microelectrodes.

Temporal Structure in Neural Activity During Working Memory

P. Mitra [in collaboration with K. Purpura and N. Schif at WMC, Cornell University; A. Polyakov, and D. Menzer, Cornell University]

Holding items in short-term memory is thought to correspond to appropriate holding patterns of electrical activity in the brain. An experimental model system much studied in this regard involves memory saccades in awake behaving macaques, where the subject remembers a target location during a memory period and performs a visual saccade to the target on an appropriate cue. Area LIP, on the lateral bank of the intraparietal sulcus, is known to encode intended saccade directions. Individual neurons may be found in this area which show a transiently elevated firing rate during the memory period of a saccade memory task, with tuning to the direction of the intended saccade.

In collaborative work with the laboratory of Richard Andersen at Caltech, we have previously discovered that in addition to the firing rates, the dynamics of the neural activity also shows tuning to intended saccade direction. We are currently studying how this tuned dynamics is represented along the cortical surface, and if the dynamics is correlated across distant parts of the circuitry known to participate in the saccade memory task. In the previous year, we have moved this research effort to the laboratory of K. Purpura and N. Schiff at the Weill Medical College of Cornell University. The setup phase of this transition is largely complete, and we are beginning to acquire data from the new location for the study.

Song Learning in the Zebra Finch

P. Mitra [in collaboration with Ofer Tchernichovski, CCNY]

The neural substrate and evolutionary origins of linguistic behavior in humans is of great biological interest, but difficult to study, since there are no other species where the acoustic communication behavior is seen to exhibit the same level of syntactic complexity. Therefore, there has been great interest in studying what has been termed the faculty of language in a broad sense, one aspect of which is the ability to learn vocalizations. This last is itself infrequently found in animals: a reason songbirds, which exhibit learning of complex vocal repertoires, have been the subject of intensive study. The species of choice in this research is the zebra finch, where all aspects of the song system have been scrutinized, ranging from the sensory-motor circuitry in the brain to the production apparatus. We have developed techniques for detailed quantification of song development, through the analysis of continuously acquired acoustic signals from birds subjected to a controlled training protocol.

In this ongoing research project, in the previous year, we have made a number of new observations. We developed a segmentation and clustering procedure that allows us to track the emergence and development of individual song elements, as well as to monitor the development of the sequential structure of these elements in the mature song. This procedure has confirmed our earlier observation that the process of song development is highly structured and may not be described by a simple minimization of an overall acoustic error. In studying the effect of age at which the song model is first presented to the learner, we have found that adult learners are able to quickly learn the acoustic structure of individual notes, but they have more difficulty with the overall sequential pattern of the song: This is apparently in contrast with the corresponding observation in humans. Sebastien Dergnaucourt, a postdoc in the Tchernichovski laboratory, has demonstrated that sleep affects song learning, particularly in the early stages; it appears that song performance degrades after night sleep during the first few weeks after exposure to the song model. This "morning deterioration" is absent in the adult bird.

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EXPERIENCE-DEPENDENT PLASTICITY OF SYNAPSES AND SYNAPTIC CIRCUITS

K. Svoboda B. Burbach, I. Bureau, V. DePaola, A. Holtmaat, A. Karpova, E. Nimchinsky, T. O'Brien, L. Petreanu, T. Pologruto, V. Scheuss, G. Shepherd, A. Sobczyk, G. Tervo, L. Wilbrecht, R. Yasuda, C. Zhang, K. Zito

The functional properties of the brain must change in response to salient sensory experiences. The nature of these changes at the level of synapses, neurons, and their networks, also known as the engram, is unknown. Our laboratory is searching for the substrates of experience-dependent plasticity in the developing and adult neocortex.

Neocortical tissue is dauntingly complex: 1 mm^3 contains nearly a million neurons, each of which connects to thousands of other neurons. To probe neurons and synapses within the intact network, we build and use sensitive tools. 2-photon laser-scanning microscopy (2PLSM) allows us to image single synapses in intact tissues and to track changes in intracellular calcium and signal transduction events. Excitation of neuronal elements by focal uncaging of neurotransmitters allows us to probe the connectivity of neural networks with high efficiency. We combine these optical methods with electrophysiological measurements of synaptic currents and potentials and molecular manipulations of neurons.

We use both in vivo measurements to address system level questions and in vitro methods to get at detailed mechanisms. As a model system we use the rodent barrel cortex. Here, whiskers are represented in a topographic manner, with information from each whisker represented by a small cortical region (barrel). Whisker maps are shaped by experience during development and reshaped in the adult. The cellular mechanisms underlying sensory map plasticity are likely to share mechanisms with those underlying learning and memory in other brain regions and other species.

[Ca^{2+}] Signaling in Single Dendritic Spines

V. Scheuss, A. Sobczyk

Long-term changes in synaptic efficacy are triggered by increases in [Ca^{2+}] in the postsynaptic neuron. We are working toward a complete understanding of the life cycle of Ca^{2+} ions in dendritic spines. We have developed a variety of assays to learn about the molecular pathways of Ca^{2+} influx, including the counting of individual Ca^{2+} permeable channels and receptors in spines. Recently, we have discovered that Ca^{2+} extrusion is inhibited by long-duration Ca^{2+} elevations, which could be an important factor underlying the induction of synaptic plasticity. In addition, we are probing the 2. messenger cascades involved with modulation of Ca^{2+} handling in small neuronal microcompartments.

Optical Studies of Single Synapses

R. Yasuda, E. Nimchinsky

Central nervous system (CNS) synapses release glutamate to activate postsynaptic channels. Activated channels can be detected at single synapses using [Ca^{2+}] imaging in postsynaptic spines. Using a novel statistical analysis of these [Ca^{2+}] signals, we counted the number of receptors opened by synaptic transmission and found this number to be small (1–5). Only a small fraction of receptors open during low-frequency synaptic transmission, and the dynamic range of synapses is large. Furthermore, stochastic interactions between ligand and receptor as well as molecular fluctuations of synaptic channels and receptors contribute to noise in synaptic transmission. Using techniques based on fluorescence energy transfer, we have begun to image the signal transduction cascades activated by synaptic transmission in dendrites.

Experience-dependent Plasticity in the Adult Cortex In Vivo

A. Holtmaat, L. Wilbrecht, V. DePaola

Sensory representations in the adult brain are stable, yet we are able to learn. What is stable and what is

plastic in the adult brain? We have succeeded in imaging dendrites, axons, and synapses over time scales of months in the *adult cortex*. We find that layer-5 dendrites and axons are stable over months. Changes in synaptic networks therefore must be local. We have imaged synaptic structure and found that a fraction of transient dendritic spines appeared and disappeared over a few days whereas other stable spines persisted for months. The stable fraction grew gradually during development and into adulthood, providing evidence that synaptic circuits continue to stabilize even in the mature brain. A direct comparison in adult mice revealed that a larger fraction of spines are stable in the visual cortex than in the somatosensory cortex, possibly reflecting differences in the capacity for experience-dependent plasticity in these brain regions. We further analyzed the ultrastructure of new spines at the level of electron microsocopy, providing evidence that synapses are formed in the adult brain. Induction of experience-dependent plasticity by manipulating the whiskers was associated with synaptic growth, suggesting that novel experiences are encoded by new synapses. Presynaptic boutons are also turning over in the adult brain with frequencies similar to those of dendritic spines.

Experience-dependent Development of Neocortical Circuits

G. Shepherd, I. Bureau, L. Petreanu

We have adopted and improved laser scanning photostimulation (LSPS) to rapidly and efficiently probe the structure of neural circuits. Sensory cortex is ordered into columns, each tuned to a specific subset of peripheral stimuli. To identify the principles underlying the construction of precise columnar architecture, we monitored the development of functional circuits in the rat barrel cortex using LSPS. Circuits impinging onto layer-2/3 neurons from layers 4 and 2/3 developed in a monotonic, precise progression. Anatomical reconstructions show that axonal arbors grow in a directed manner. We find little evidence for transient hyperinnervation at the level of cortical columns. Consistently, synaptic currents measured in layer-2/3 neurons just after these neurons cease to migrate (PND 8), revealed already spatially well-tuned receptive fields.

We used LSPS to determine the locus of experience-dependent plasticity. Layer-2/3 neurons differed in their spatial distributions of presynaptic partners: Neurons directly above barrels received, on average, significantly more layer-4 input than those above the septa separating barrels. Complementary connectivity was found in deprived cortex: Neurons above septa were now strongly coupled to septal regions, whereas connectivity between barrel regions and layer 2/3 was reduced. These results indicate competitive interactions between two thalamocortical circuits, the VPM-barrel and POm-septal pathways, in the establishment of precise intracortical circuits. We are beginning to apply these techniques to test for the role of inhibitory circuits in experience-dependent plasticity.

Cellular and Molecular Mechanisms of Dendritic Spine Morphogenesis

K. Zito, C. Zhang

Most of the excitatory synapses in the cortex occur on dendritic spines, small protrusions extending from neuronal dendrites. Spines are highly motile during development, suggesting a role for spine dynamics in synapse formation. We searched for molecules that altered the dynamics of spine morphogenesis by examining the effects of overexpression of wild-type, activated, and dominant negative versions of candidate genes on spine number, density, morphology, and dynamics. We are studying Neurabin-I (Nrbl), a *neu*ronal-specific *a*ctin-*bin*ding protein. Nrbl is a 122-kD protein that contains an amino-terminal actin-binding domain, a protein phosphatase 1 (PP1)-binding site, a PDZ domain that binds p70S6K, and a carboxy-terminal coiled-coil dimerization domain. Green fluorescent protein (GFP)-tagged Nrbl localized specifically to dendritic spines in neurons in slice culture, and this localization required an intact actin-binding domain. Remarkably, a severely truncated GFP-Nrbl caused a dramatic increase (more than twofold) in the number of spines. Using electron microscopy, we found that Nrb is concentrated at sites of cell-cell contact and that morphological changes reflect an increase in the number of synapses.

FIGURE 1 Coronal section through one olfactory bulb of a transgenic mouse expressing a modified VAMP protein and GFP. The bulb-like structures on the circumference are individual glomureli, the termination zones of receptor neurons expressing particular olfactory receptors.

Molecular Methods to Reversibly Inactivate Synapses In Vivo

A. Karpova, G. Tervo, B. Burbach

A major roadblock in gaining an understanding of neural networks is our inability to modulate selected circuit elements in vivo with temporal control and specificity. We have begun a research program to harness recent insights about the molecules that regulate neurotransmitter secretion to design genetically deliverable systems that can interfere with synaptic function in a rapidly inducible manner. Interference is achieved with dimerization induced by rapamycin-like compounds. The first two systems, based on synaptophysin and VAMP, are currently being tested in in vitro systems. One system based on VAMP has also been targeted to specific subsets of neurons in the olfactory bulb (Fig. 1) and in the cerebellum for testing in the intact brain.

Genetically Encoded [Ca^{2+}] Indicators

T. Pologruto

A major breakthrough in functional imaging was the development of genetically encoded Ca^{2+} indicators based on fluorescent proteins. These indicators couple Ca^{2+} binding to a calmodulin domain to a conformational change in the fluorescent protein and an optical signal. Because of the complicated nature of Ca^{2+} binding to calmodulin in the presence of calmodulin substrate, the dynamics of these indicators are not understood. We express genetically encoded indicators in brain slices and explore their responses to physiological Ca^{2+} concentration changes in neurons. We combine these measurements with quantitative Ca^{2+} imaging in a second fluorescence channel using synthetic Ca^{2+} indicators. This will allow us to construct detailed kinetic models of genetically encoded indicators. These studies will also teach us about the signals activating calmodulin in intact neurons.

Instrumentation

K. Svoboda, T. Pologruto, T. O'Brien, R. Yasuda
[in collaboration with R. Eifert, Cold Spring Harbor Laboratory]

Our hardware development efforts during the past year have focused on developing new approaches to studying signaling in synaptic microcompartments. We have developed a microscope combining 2-photon microscopy with fluorescence lifetime imaging (FLIM). FLIM facilitates quantitative imaging of fluorescence resonance energy transfer (FRET), allowing detection of protein-protein interactions in situ. We have also set up a microscope that allows simultaneous 2-photon imaging and uncaging with single synapse resolution. We have also written software to control a laser-scanning microscope with two lasers (one for imaging and the other for uncaging) and FLIM detection.

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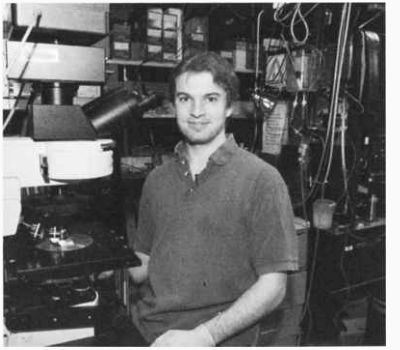

Gowan Tervo

NEUROGENETICS OF MEMORY IN *DROSOPHILA*

T. Tully J. Barditch, F. Bolduc, B. Chen, L. Grady, J. Kui, C. Li, J. Luo, J. O'Neil, S. Rachakondra, M. Regulski, S. Xia

We are using the fruit fly model system to discover genes involved in memory formation. Given its economy of scale (simpler genome and nervous system, rapid generation cycle, cheap husbandry) and the genetic tools available, this model system offers the possibility to identify a large part of the gene network that participates in this complex emergent property of the animal. Enough past work already has established that similar genes likely will participate in mammalian memory formation. Thus, our gene discovery in *Drosophila* represents the first step of a broader experimental strategy to delineate the genetic basis of cognitive (dys)function in humans.

MUTANTS AND MICROARRAYS

Past studies of single-gene mutants have suggested a genetic dissection of memory formation into five distinct temporal phases: initial acquisition or learning (LRN) of an odor–foot shock association, short-term memory (STM), middle-term memory (MTM), anesthesia-resistant memory (ARM), and long-term memory (LTM). In essence, any given mutation yields a primary disruption of just one memory phase, sometimes with secondary disruptions of temporally "downstream" phases. Further evidence for the biological validity of these memory phases accumulates. In collaboration with Dr. Minoru Saitoe and co-workers at the Tokyo Metropolitan Institute, for instance, we recently have shown that age-associated memory impairment (AAMI) results specifically in the loss of MTM. Consistent with this notion, AAMI does not occur in *amnesiac* mutants, in which MTM is defective.

Evidence for functionally independent memory phases also is clear. Mutation of the CREB gene disrupts LTM without affecting ARM, for instance, whereas mutation of the *radish* gene disrupts ARM without affecting LTM. These observations argue that distinct biochemical pathways or anatomical sites underlie the various memory phases. Our recent gene discovery has begun to clarify these alternative hypotheses (see below).

We have identified 60 new memory mutants from a behavioral screen, which define 51 new genes. This approach is designed to identify genes that function either during development to give rise to relevant brain structures or during memory formation to give rise to the physiological response. Such mutations may exert their effects anywhere within the five phases of memory formation. A limitation of this approach, however, is that the mutations must yield homozygous-viable adults, thereby preventing the identification of any essential genes. We also have identified 3971 candidate memory genes (CMGs) from DNA microarray experiments. These CMGs are differentially regulated in normal flies subjected to spaced versus massed training. Previously, genetic experiments have established that the only difference between these two training protocols is the induction of transcription-dependent LTM by the former. This approach is designed to identify essential or nonessential genes involved specifically in the physiology of LTM formation, and it can reveal the involvement of essential genes. Strikingly, 17 of the memory mutants carry molecular lesions in or near CMGs from microarray experiments.

This convergence of our two approaches identified the *pumilio/staufen* pathway to be involved in memory formation. This pathway participates in the subcellular compartmentalization of mRNAs and the regulation of their translation locally. We hypothesize that the *pumilio/staufen* pathway participates in modifications of specific synapses during memory formation. Continued focus on these convergent data promises the identification of further novel cellular processes involved in behavioral plasticity.

CIRCUITS AND PHASES

This work was done in collaboration with Dr. A.-S. Chiang (Tsing Hua National University, Taiwan) and Dr. J. Dubnau here at CSHL. These 60 new memory mutants were generated using "enhancer-trap" transposons—jumping genes that not only disrupt the function of endogenous target genes, but also appropriate

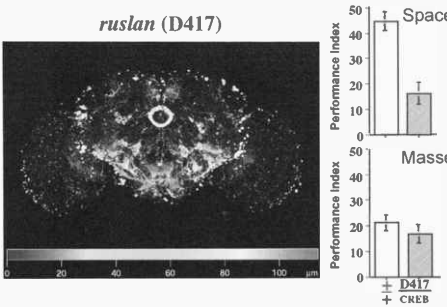

FIGURE 1 CREB-dependent long-term memory formation occurs in an anatomically distinct site outside mushroom bodies. (*Left panel*) The *ruslan* enhancer-trap expression pattern was revealed with green fluorescence protein (GFP) reporter gene. GFP appears in a relatively complex anatomical pattern, which includes antennal lobes, subesophogeal ganglia, and the central complex. Notably, GFP is not expressed in mushroom bodies, a predominantly anatomical region of the insect brain previously known to participate in olfactory memory formation. (Coding refers to depth of confocal optical sections in whole-mount adult brain.) (*Right panels*) Expression of a repressor form of CREB (CREB) was driven by various enhancer-trap transposon insertions, including *ruslan* (D417). In such transgenic flies (D417/CREB), 1-day memory after spaced training (Spaced) was disrupted, whereas 1-day memory after massed training (Massed) was similar to that in normal flies (+/+). This outcome is the characteristic signature of specific disruptions of LTM. In contrast, expression of CREB repressor in enhancer-trap lines showing preferential expression in mushroom bodies had no effect on 1-day memory after spaced training (data not shown). Since *ruslan* does not express in mushroom bodies, these data indicate that LTM forms at an anatomical site distinct from mushroom bodies.

the target genes' endogenous enhancers to drive expression of a reporter gene. With such a genetic tool, we were able to visualize the neural circuits that expressed each of the "memory genes." Many of these genes showed relatively complex patterns of expression in various brain anatomies, including the mushroom bodies—neuropillar structures already known to be required for the proper formation of olfactory memory. Some memory genes, in contrast, showed patterns of expression that did not include mushroom bodies, thereby suggesting a broader neural circuitry involved in olfactory memory. One of these informative memory mutants is *ruslan* (Fig. 1).

In collaboration with Dr. Carla Margulies in Jerry Yin's lab here at CSHL, we have used these enhancer-trap lines to express a repressor form of CREB (CREB-r) in different anatomical regions of the brain. In this manner, we are identifying anatomical regions of the brain that may or may not participate in the formation of LTM. Much to our surprise, expression of CREB-r in mushroom bodies did not disrupt LTM (data not shown). Instead, expression of CREB-r by the *ruslan* enhancer-trap disrupted LTM specifically (Fig. 1). Importantly, the *ruslan* expression pattern does not include mushroom bodies, thereby indicating that LTM forms outside of mushroom bodies, even though LRN and early memory (STM and MTM) require normal mushroom body function. More generally, these data suggest a "transference" of memory over time from one anatomical region of the brain to another—a phenomenon also known to exist in humans.

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LONG-TERM MEMORY FORMATION IN *DROSOPHILA*

J. Yin K. Ando, E. Drier, V. Gupta, J. Horiuchi, C. Margulies, E. Paniagua, P. Wu, H. Zhou

The Biology of DaPKM

E. Drier, E. Paniagua, J. Yin

In neurons, the truncated form of DaPKC (DaPKM) has been shown to be a critical player in memory formation and synaptic plasticity. Is the truncated isoform unique to neurons? We have isolated 45 new imprecise excisions of the l(2)k06403 P-element insertion. This P-element is inserted into the promoter region of Transcript B, which codes for one of the DaPKM isoforms (DaPKMp60). Complementation analysis shows that these new insertions complement both the original P-element mutation and the deficiency that uncovers the *DaPKC* gene. Phenotypic characterization of homozygous mutations show that the excisions can be grouped into classes that affect fertility, ranging from a wild-type mother to mutants that are mildly or severely affected for egg laying. Western analysis of ovaries shows that there is an inverse correlation between the severity of the mutation's oogenesis phenotype and the amount of a DaPKMp45 protein. The different classes of mutants make similar amounts of full-length protein (DaPKCp73). Mutations that are mildly affected result in almost wild-type levels of the protein, whereas mutants that make little or no protein are severely compromised. These data establish the importance of DaPKM isoforms in oogenesis, and suggest that the p45 isoform, which is barely detectable in head extracts, participates in oogenesis. Light microscopic analysis of ovaries from mutant mothers suggests that the excisions affect a relatively early stage in oogenesis, prior to Stage 10. It will be interesting to determine if the decrease in DaPKMp45 is somehow disrupting a maintenance function in one of the localization processes that is important in oogenesis, consistent with our interpretation of DaPKMp60's function in neurons.

DaPKM and Synaptic Tagging

P. Wu, H. Zhou, J. Yin

We have continued to analyze proteins that interact with DaPKMp60 and DaPKMp47 in head extracts. When fly head extracts are made, there are three predominant protein isoforms that we can detect: DaPKCp73, DaPKMp60, and DaPKMp47. These can be separated using traditional biochemical approaches. When a partially enriched extract is run over Superose 6 gel-filtration columns, DaPKMp60 and DaPKMp47 are found in fractions that contain different-sized protein complexes. The DaPKMp47-containing fraction is larger and runs consistent with a 440-kD complex. The DaPKMp60-containing fraction runs around 150 kD. An interesting array of proteins can be found in the 150-kD fraction, including Dlg, SAP97, dFXR, and other proteins that are connected with RNA metabolism and translational regulation. These proteins are known to interact with DaPKC in other developmental contexts (oogenesis, asymmetric cell division, cell polarity) and are consistent with subcellular functions involving receptor trafficking, localized translation, and cytoskeletal rearrangements. The same proteins are found in a DaPKMp60-containing fraction that is eluted from an antibody affinity column. This argues that many of these proteins may actually interact with DaPKMp60, since they coelute on gel-filtration and affinity chromatography. Interestingly, the 440-kD fraction that contains DaPKMp47 does not contain any of these proteins, suggesting isoform specificity in the interactions. We are continuing to purify these fractions in anticipation of obtaining fractions clean enough for mass spectrometry.

Functional Anatomy of Long-term Memory Formation

C. Margulies, V. Gupta, J. Yin [in collaboration with A.-S. Chiang and T. Tully, Cold Spring Harbor Laboratory]

The traditional Gal4/UAS bipartite system has been used to ask where *dCREB2-b* expression results in effects on long-term memory (LTM), but not learning or memory after massed training. A number of the commonly used driver lines that express in the mushroom body do not result in any effects on LTM formation, although pan-neuronal or ubiquitous expression of the particular *dCREB2-b* transgenes specficially affect memory after spaced training. Using a number of driver lines that resulted from the Hartford screen, and which do not express at very significant levels in the mushroom bodies, LTM can be specfically disrupted. Because it is difficult to know if the mushroom bodies have a higher threshold for *dCREB2-b*-mediated disruption, or if chronic expression of the *dCREB2-b* transgene allows compensation during development, it is difficult to conclude that the mushroom bodies are not needed for LTM formation. The spatial pattern of expression has been examined using high-resolution treatment of head tissue, green fluorescent protein (GFP) expression and confocal microscopy, and the emerging impression is that the anatomical regions required for LTM formation may lie outside of the mushroom bodies. However, use of the tetracyline-inducible system will be needed to definitively rule in other anatomical regions, and perhaps help rule out the involvement of the mushroom bodies in LTM formation.

dCREB2 and Neurodegeneration

K. Ando, J. Yin

Huntington's disease results from a polyglutamine expansion in the coding region of the *huntingtin* gene. Recently, it has been shown that expanded polyglutamine runs interfere with the response-element-binding protein (CREB)-binding protein (CBP) and CREB-mediated transcription in cells. We find that polyQ-expanded flies are compromised for viability and have decreased *dCREB2* activity and that this lethality is sensitive to the gene dosage of the *dCREB2* gene. When the S162 (loss-of-function) mutation is combined with polyQ-expressing transgenes, toxicity is increased. Genomic transgenes of *dCREB2* partially suppress the lethality in a dose-dependent manner, suggesting that the effects of polyQ can be reversed by increasing *dCREB2* activity. This is consistent with the interpretation that much of the effects of polyQ in flies occur at the transcriptional level. Transgenes that overproduce PKA also suppress both lethality and the effects that polyQ have on *dCREB2* activity, suggesting that it is also suppressing through the same pathway as dCREB2-b and CBP. The ability of this single pathway to partially suppress the toxic effects of polyQ expansion and overproduction has interesting therapeutic implications.

Regulation of dCREB2 Activity

J. Horiuchi, J. Yin [in collaboration with R. Kwok, University of Michigan]

Previous work showed that dCREB2 is regulated at the level of DNA binding and that casein kinase phosphorylates one or more sites in the KID domain of the protein to inhibit binding. These potential casein kinase sites are conserved on mammalian CREB, and the protein is susceptible to the same regulation. In mouse tissue, we detect two pools of protein, one that contains unphosphorylated CREB, and fits the *Scientific American* view of the protein and the other that is phosphorylated and inhibited from binding DNA. This latter pool may not require S133 phosphorylation for transcriptional activation and may instead use a coactivator which does not require S133 phosphorylation for protein:protein interaction. It is unknown if there are ever two comparable pools in *Drosophila*. In the steady state, we can only detect protein totally phosphorylated on both S231, the *Drosophila* equivalent to S133, and protein phosphorylated on one or more of the putative casein kinase sites.

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NEURAL SUBSTRATE OF SELECTIVE AUDITORY ATTENTION IN THE CORTEX

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T. Hromadka S. Rumpel M. Wehr

We use a combination of theoretical and experimental approaches to study how the brain computes. The brain is able to solve hard computations that remain far beyond the reach of the fastest computers. Our goal is to understand this computation at the synaptic, cellular, behavioral, and algorithmic levels.

One example of such a hard computation is the "cocktail party problem." When we tune in to one voice at a cocktail party, and tune out the others—a task that remains beyond the capacity of modern computers—we are engaging in a form of selective auditory attention. Our ability to attend selectively is not limited to the auditory domain: Analogous tasks demand selective attention in the visual and even somatosensory domains. In monkeys, visual attention selectively enhances neural activity even in the earliest stages of visual cortical processing. This enhancement is surprising because the areas associated with these first stages of visual processing have traditionally been thought of as representing the sensory world faithfully, in a way that depends only on the properties of the sensory input itself. The discovery of attentional modulation overturns the notion that the peripheral sensory cortex is a passive "TV screen" available for viewing by a "homunculus" buried deep within the cortex.

The specific projects in our laboratory fall into two main categories. First, we are interested in how neurons represent auditory stimuli, and how these representations are computed from the cochlear inputs half a dozen synapses away. To address these questions, we are using electrophysiological and imaging approaches in anesthetized rats as well as computational approaches to characterize the properties of natural sounds. Second, we are interested in how these representations are modified dynamically—within seconds—in awake behaving rats by the demands imposed by attentional tasks.

Variability of Coding in the Auditory Cortex

M. Deweese

Computers rely on extremely precise, low-noise components to compute, whereas the components that make up neural circuits appear to be very noisy. Nevertheless, brains outperform computers on the kinds of hard computational problems required for survival in the real world. To understand how brains compute in the presence of such high levels of apparent noise, we are characterizing the sources of variability (i.e., noise) in single neurons. Using in vivo whole-cell patch-clamp recording techniques, we are examining the trial-to-trial variability of the postsynaptic potential (PSP) elicited by brief tone pips. In some neurons, trial-to-trial variability in the PSP is small, consistent with "private" sources limited to only the neuron under study; but for other neurons, "shared" sources of variability produce circuit-wide fluctuations in the synaptic drive to the neuron and its neighbors, greatly increasing the apparent noise in the PSP. These stimulus-independent correlations could provide a substrate for feedback underlying cognitive processes, such as attention and motivation.

In Vivo Whole-cell Patch-clamp Recordings of Sound-evoked Synaptic Responses in Auditory Cortex of Awake Restrained Rats

M. Deweese, T. Hromadka

Neurons in the auditory cortex respond to some sounds but not to others. What determines this selectivity? We are using whole-cell patch-clamp recording methods in vivo to measure the synaptic currents elicited by simple and complex auditory stimuli. Patch-clamp recordings provide a much richer source of information than do conventional single-unit extracellular recordings because they allow us to monitor not just the *output* of the neuron—the spike train—but the input as well. These data provide clues about the representations with which the cortex solves hard problems in auditory processing.

Optimal Stimulus for Auditory Cortex

T. Hromadka [in collaboration with M. Chklovskii, Cold Spring Harbor Laboratory]

Single neurons in auditory cortex respond differently to different sounds. What are the sound features that evoke the strongest response? To answer these questions, we use in vivo cell-attached recordings in awake Mongolian gerbils in combination with evolutionary algorithms to search for the most appropriate stimulus for the individual neurons. In addition, we are characterizing the responses of single neurons to a wide variety of conventional stimuli, including pure tone pips, sweeps, and moving ripples.

Separation of Sound Sources by Awake Behaving Animals

G. Otazu, L.-H. Tai

Sounds in the natural world rarely occur in isolation, but rather as part of a mixture. To survive, the auditory system must be able to selectively attend to one sound source and ignore others—and it does so more effectively than any artificial system yet devised. To understand how this is performed, we are using multi-electrode recording (tetrode) technology to monitor the activity of many neurons simultaneously in awake, behaving rodents performing an auditory discrimination task.

Analysis of AMPA-R Trafficking during Learning-induced Reorganization of the Auditory Cortex

S. Rumpel [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

Does learning change the strength of neuronal connections in the brain? Insertion of new postsynaptic AMPA (α-2-hydroxy-5-methyl-4-isoazole) receptors (AMPA-R) has been identified as a major process leading to increased synaptic strength. However, these results have been obtained primarily in cultured neurons, and the relationship of these processes to learning in the intact animal has remained unclear. We have been investigating whether reorganization of the auditory cortex induced by classical conditioning leads to increased insertion of AMPA-Rs. We are using virus-mediated overexpression of specially engineered recombinant AMPA-Rs as a marker of this in vivo plasticity. These properties will enable us to detect added recombinant receptors after a learning protocol and thereby establish the role of AMPA insertion in vivo. If the molecular rules of learning identified in cultured neurons should apply in vivo, we will also have developed a valuable tool for identification of synaptic circuits involved in memory formation.

Responses to Complex Stimuli

M. Wehr [in collaboration with C. Machens and C. Brody, Cold Spring Harbor Laboratory]

How do neurons in the auditory cortex encode complex stimuli, such as animal calls or the sound of rain falling on leaves? Sensory physiologists often ignore this question, dismissing it as too difficult to approach in a systematic fashion. Instead, they often limit their inquiries to simple stimuli, such as (in the auditory system) brief tone pips or sweeps. We have instead tackled this problem head-on by measuring the responses of neurons in the auditory cortex to a wide variety of natural sounds. We then use these responses to construct and test models of how cortical neurons encode natural sounds.

Inhibitory and Excitatory Contributions Underlying Synaptic Responses

M. Wehr

The cortical responses to sensory stimuli such as sounds consist of a mix of excitation and inhibition. Conventional extraceullular recording techniques using tungsten electrodes provide information only about excitatory responses. We have developed an approach using whole-cell patch-clamp recording in vivo in voltage clamp mode to tease apart the temporal dynamics of the excitatory and inhibitory drive underlying responses to auditory stimuli.

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong H.-F. Guo K. Iijima
I. Hakker A. Mamiya
F. Hannan Y. Wang
I. Ho

We are interested in the neural basis of learning and memory and are taking an approach of combining functional analyses and genetic manipulation in the study of *Drosophila*. Currently, we are pursuing two major projects. First, we are establishing *Drosophila* models for studying genes involved in human neurodisorders that impair learning and memory. In particular, we are interested in genes known to contribute to neurofibromatosis 1 (*Nf1*) and Alzheimer's disease. *Nf1* patients are identified by neurofibromas and other symptoms, including learning defects. We are investigating a hypothesis that the tumor-suppressor gene *Nf1* not only acts as a Ras-specific GTPase-activating protein (GAP), but also is involved in mediating G-protein- and Ras-stimulated activation of adenylyl cyclase (AC); NF1-dependent AC pathways are required for learning and memory. In the case of Alzheimer's disease, our study has been limited to examining how presenilin and accumulation of Aβ affect the age-dependent decline of learning ability. This approach, on the one hand, serves as a new way to identify biochemical cascades underlying learning and memory in *Drosophila*. On the other hand, it may also provide insights into pathogenesis of the diseases. Second, we are investigating how odors are encoded by population neuronal activity in the fly brain and will ultimately study learning and memory at the level of population neural activity. Such study is carried out in living flies via optical recordings. The specific projects are described below.

Genetic Dissection of NF1-regulated Pathways in *Drosophila*

F. Hannan, I. Ho, I. Hakker

Neurofibromatosis type I (NF1) is an inherited disorder which affects 1 in 3500 people worldwide. A large number of different mutations are seen in NF1 patients, including some changes that only alter a single amino acid out of the whole NF1 protein, yet still result in NF1 disease. Several of these missense mutations have been reconstructed in our lab and introduced into *Nf1* mutant *Drosophila*. These mutated human NF1s can overcome some cAMP-dependent defects in *Nf1* mutant flies, including small body size and learning defects, indicating that the cAMP pathway is not affected by these mutations.

Two mutations in the GAP-related domain (GRD), however, show defective long-term memory even though learning is normal in these flies. This suggests that learning and memory may be differentially regulated in flies by NF1 acting through the cAMP and Ras pathways, respectively. Verification of this hypothesis will provide important insights into the mechanism whereby NF1 affects learning. We propose to generate further missense mutants and some large deletion mutants, and look at their effects in *Nf1* mutant flies, to try to pinpoint the region(s) associated with cAMP activity versus Ras activity.

We have also recently identified a novel pathway for NF1-dependent adenylyl cyclase (AC) activation in flies that involves growth factor stimulation of Ras. This pathway requires both Ras and NF1 activity to stimulate cAMP production. A second pathway requiring NF1 but not Ras activates the rutabaga AC which is critical for learning in flies. The mutant human NF1s provide an important resource for studying the role of NF1 in this new pathway because many other components of the pathway cannot be fully knocked out in flies since they have lethal effects.

Finally, the new technology of DNA microarrays has allowed us to look at all of the genes in *Drosophila* and identify about 100 genes whose expression appears to be controlled by NF1 activity. The expression of many of these genes is also affected by human NF1 when it is expressed in flies. We hope to look at flies expressing mutated human NF1s to see if there are any differences that may be related to the pathway affected by the mutations.

The basic research ideas proposed here promise to discover and delineate new pathways and proteins both upstream and downstream from the NF1 protein in *Drosophila* and should stimulate experiments in mammals to verify the generality of these pathways. Ultimately, this should lead to new ideas for treatment strategies that may be of direct benefit to NF1 patients.

A *Drosophila* Model of Alzheimer's Disease

K. Iijima

Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by the progressive decline in memory accompanied by histological changes, including neuronal loss and the formation of neurofibrillary tangles (NFTs) and senile plaques (SPs). The accumulation of $A\beta_{42}$ peptide, the major component of SPs, has been hypothesized as the primary event in AD pathogenesis. Multiple pathogenic mutations have been identified in β-amyloid precursor protein (APP), Presenilin1 (PS1), and Presenilin2 (PS2) genes in familial AD (FAD), and all of these mutations caused excessive accumulation of $A\beta_{42}$. However, it has not yet been shown in any animal models that the accumulation of $A\beta_{42}$ is sufficient to induce AD phenotypes.

We used *Drosophila* as a model organism to address this question. To determine whether *Drosophila* can be used as a model to study the molecular basis of AD pathogenesis, we examined the effects of $A\beta_{42}$ in the *Drosophila* brain using the GAL4-UAS system. The expression of $A\beta_{42}$ in the fly nervous system led to the formation of diffused amyloid deposits, age-dependent learning defects, locomotor disability, extensive neurodegeneration, and premature death. These results strongly suggest that excessive accumulation of $A\beta_{42}$ is sufficient to cause memory defects and neurodegeneration resembling AD and that the molecular basis underlying the Aβ toxicity is conserved over different organisms. Using this fly AD model, we are currently conducting genetic and pharmacological screening for AD therapeutics targeting Aβ-induced neurotoxicity and Aβ catabolism, as well as for understanding the molecular and cellular basis of AD pathogenesis.

Stereotyped Representation of Odors in Mushroom Bodies

Y. Wang, A. Mamiya

To study the representation of olfactory information in higher brain centers, we have developed transgenic flies that express the green fluorescent protein (GFP)-based Ca^{++} sensor, G-CaMP, specifically in the mushroom body (MB), a higher olfactory center known to have an important role in olfactory memory formation. With the help of two-photon microscopy, we were able to examine how the MB neurons (Kenyon cells) respond to odor stimulation at a single cell resolution. Odors evoked large fluorescence transients in the somata of MB neurons (Kenyon cells) and in the calyx, the neuropil of the MB. According to their response to different concentrations of an odor, we were able to classify Kenyon cells into two groups: one with a broad receptive concentration range that spanned several magnitudes and one with a narrow receptive range. The latter group responded only to a specific concentration of an odor. Different odors appeared to activate different subsets of Kenyon cells. The spatial distributions of these cells seemed to be conserved across individuals for each odor. In some cases, a Kenyon cell activated by an odor could be found in similar locations of the brain in different flies with a precision of the size of one or two Kenyon cell somata. These results indicate a stereotyped distribution of odor-evoked activities in the MB.

Although 2-photon imaging using G-CaMP allows monitoring of the activity of multiple neurons at a very high spatial resolution, it lacks temporal resolution. We have begun to combine electrophysiological recordings with the imaging in order to study the odor-evoked activities at a much higher temporal resolution. The combination of electrophysiological recordings and imaging should help provide a better understanding of how the olfactory information is processed at higher olfactory centers.

Role of Akt in Synaptic Plasticity and Learning

H.-F. Guo

Long-term changes in synaptic function, such as long-term potentiation (LTP) and long-term depression

(LTD), are widely believed to be the cellular mechanism for refining neural circuits and for learning and memory. Serine/threonine protein kinases, such as PKA, PKC, CAMKII and MAPK, have critical roles in long-term synaptic plasticity. Akt, known as PKB, is one of the major targets of the PI-3 kinase activated by signaling from Ras and multiple receptor tyrosine kinases, such as the insulin receptor. The activation of Akt leads to phosphorylation of an array of target proteins mediating diverse physiological functions. Numerous studies have focused on the essential role of Akt in glucose metabolism, anti-apoptosis, and cell proliferation and cell survival, including neuronal survival. Accumulating evidence, however, suggests that Akt may also have a function in the synaptic physiology. We examined the function of Akt in synaptic transmission and synaptic plasticity at the *Drosophila* neuromuscular junction (NMJ).

The NMJ is the only preparation in *Drosophila* available for quantitative analysis of synaptic transmission at identifiable synapses. It has been extensively used for studying functions of proteins involved in synaptic vesicle release and genes involved in learning and memory. However, genetic analyses have been limited to short-term plasticity on this preparation; long-term synaptic plasticity, such as LTP and LTD, has not been shown. We characterized a form of long-term synaptic plasticity, long-term depression (LTD), at the *Drosophila* larval NMJ. We found that Akt is specifically involved in mediating LTD, but not short-term synaptic plasticity. We also found that olfactory learning is disrupted in flies, and the learning defect is rescued by induced expression of *akt* transgene. Therefore, Akt is required for mediating both long-term synaptic plasticity and learning in *Drosophila*. We will characterize the role of Akt-related signaling in synaptic plasticity and learning and memory.

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PLANT DEVELOPMENT AND GENETICS

Like animals, plants use unspecialized stem cells to create new organs and tissues. One question fundamental to all multicellular life is how organisms trigger stem cells to proliferate and subsequently adopt special properties characteristic of a particular organ or tissue, a process called stem cell patterning. CSHL scientists are investigating the signals plants use to guide stem cell patterning. Their findings are shedding light on some processes that are common to plant and animal development, and on others—unique to plants—that could ultimately have significant impacts on agriculture, horticulture, and forestry.

One mechanism that appears to be involved in stem cell patterning, and that is unique to plants, is the movement of proteins between cells through structures called plasmodesmata. David Jackson and his colleagues have created several methods for investigating how proteins move through plasmodesmata to carry out stem cell patterning. That understanding could also help block the spread of devastating plant viruses, which travel along the same intercellular highways. Additionally, they have created a genome-wide method to help reveal the locations and functions of some 7000 proteins encoded by the genome of the mustard relative *Arabidopsis*, a powerful model organism for the study of plant growth and development.

Genes and proteins studied by Marja Timmermans' lab are also emerging as key players in guiding stem cell patterning and organ development. She has identified several proteins that act in a complex way to turn key genes on or off at the correct time and in the correct location as plants develop, a process that is required, for example, for the proper outgrowth of leaves and for differentiating the top layers versus the bottom layers of leaves. Timmermans' lab has also uncovered evidence that leaf patterning is controlled by RNA interference (see below).

Part of Rob Martienssen's interest in exploring plant genetics and development has focused on a specialized type of DNA structure called heterochromatin. By virtue of the fact that heterochromatin is typically rich in "transposable elements" (segments of DNA that usually stay put but can move to other chromosomal locations), most often stays tightly packaged, and has few canonical protein-coding genes, heterochromatin has a unique impact on chromosome structure and function. Martienssen's lab is exploring the activity of genes located within heterochromatin and how such genes are silenced through the influence of transposable elements (or ancient remnants of such elements). In both plants and yeast, the lab has found that RNA molecules transcribed from remnants of transposable elements trigger the formation of heterochromatin, and hence establish gene silencing, through RNA interference.

PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen J.M. Arroyo, M. Byrne, D. Goto, C. Kidner, Z. Lippman, N. Marinsek, B. May, P. Rabinowicz, D. Roh, M. Ronemus, J. Simorowski, R. Shen, A. Tang, U. Umamaheswari, M. Vaughn, E. Vollbrecht, T. Volpe

Epigenetic mechanisms regulate transposon silencing, gene control, and stem cell function in plants, which provide a useful model for higher eukaryotes. Using *Arabidopsis* chromosome-4 microarrays, we have shown that small RNA and chromatin remodeling regulate heterochromatin through transposons. In fission yeast, RNA interference (RNAi) of centromeric transcripts regulate chromosome behavior via histone modification, and we have found similar transcripts in plants. Stem cell function in plants depends on *asymmetric leaves1, bellringer, ramosa1*, and *argonaute*. *Argonaute* has an important role in RNAi, and we have demonstrated that spatially restricted small RNA can guide leaf polarity. During the year, we were joined by postdoc Derck Goto, as well as by our URP Nina Marinsek. We said goodbye to Erik Vollbrecht and Tom Volpe who left Cold Spring Harbor for faculty positions at Iowa State and Northwestern.

Transposable Elements, Epigenetic Control, and the Origin of Heterochromatin

Z. Lippman, M. Vaughn, B. May, and R. Martienssen [in collaboration with W.R. McCombie and V. Mittal, Cold Spring Harbor Laboratory; J. Carrington, Oregon State University; R.W. Doerge, Purdue University; and V. Colot, INRA/CNRS Evry, France]

Heterochromatin is a major component of the genome that remains condensed throughout the cell cycle and influences gene expression. Heterochromatin in *Arabidopsis* is derived from euchromatin by insertion of transposable elements (TEs) and related tandem repeats. We have used genomic tiling microarrays to profile heterochromatic modifications such as DNA methylation and histone H3 Lys-9 dimethylation (H3mK9), and we have found that these marks are restricted to TEs. TEs are distinguished from genes by the chromatin remodeling ATPase *DECREASE IN DNA METHYLATION 1* (*DDM1*). Small interfering RNAs (siRNAs) correspond to TEs and repeats, providing a basis for their distinction. Genes are mostly insulated from the silencing effects of heterochromatin, but TEs can control genes epigenetically when inserted within them. The euchromatic, imprinted gene *FWA* resembles such genes in that its promoter is provided by a TE which contains tandem repeats associated with siRNA and is silenced epigenetically by *DDM1* and the DNA methyltransferase *MET1*. Thus, TEs and related repeats define heterochromatin and likely have major regulatory roles in repeat-rich genomes.

Arabidopsis Centromeric Repeats Are Transcribed

B. May, Z. Lippman, R. Martienssen

Centromeres are responsible for the inheritance of a complete set of chromosomes following cell division, via kinetochore assembly and spindle attachment. In plants and animals, centromeres comprise thousands of short, tandem 150–180-bp repeats with interspersed retrotransposons. Pericentromeric repeat sequences differ between closely related species, indicating that chromatin rather than DNA sequence determines kinetochore assembly. As in fission yeast, the centromeric repeats of *Arabidopsis* are transcribed and give rise to small RNA via RNA interference (RNAi). By examining centromeric chromatin in *Arabidopsis* mutants defective in DNA methylation, histone modification, and RNAi, we have found that the centromeric repeats differ in their regulation. Some are silenced by DNA methyltransferase *MET1* and the chromatin remodeling ATPase *DDM1*, whereas others are controlled by RNAi genes *DCL1* and *AGO1*, the histone methyltransferase *KYP1*, and the DNA chromomethylase *CMT3*. Thus, centromeres may be composed of subdomains that are transcriptionally as well as posttranscriptionally regulated.

Presetting and Methylation of Transposable Elements Is Associated with Small RNA and Histone Modification

Z. Lippman, B. May, R. Martienssen

The inheritance of active (preset) transposons is a classical model for epigenetics first studied by Barbara McClintock in maize. In *Arabidopsis*, mutants in the DNA methyltransferase *MET1*, the chromatin remodeling ATPase *DECREASE IN DNA METHYLATION1* (*DDM1*), and the histone deacetylase gene *SILENCING1* (*SIL1*) activate most transposons with a concomitant loss of both DNA methylation and H3mK9. In contrast, mutations in the H3mK9 methyltransferase *KRYPTONITE* (*KYP*), the RNAi gene *ARGONAUTE* (*AGO1*), and the CNG methyltransferase *CHROMOMETHYLASE* (*CMT3*) regulate only a subset of transposons. Following backcrosses with wild type, activated transposons were inherited from *met1* and *ddm1*, which lost corresponding siRNA unlike the other mutants. This indicates a role for siRNA in de novo silencing by *MET1* and *DDM1*. Thus, two distinct mechanisms are involved in transposon silencing via siRNA: one involving *DDM1, MET1*, and *SIL1*, and the other involving *KYP* and *AGO1*, that directs DNA methylation via *CMT3*.

Genomic Consequences of RNAi in Fission Yeast

T. Volpe, D. Goto, N. Marinsek, R. Martienssen [in collaboration with T. Cech, University of Colorado; J. Bahler, Sanger Centre, U.K.; and G. Thon, University of Copenhagen]

We previously demonstrated that RNAi of centromeric repeat transcripts was required for chromatin modification and centromere function in fission yeast (Volpe et al., *Science 297:* 1833 [2002]). We have extended these studies to the entire genome using microarrays. All three mutants in RNAi, *ago1*$^{-}$ (arg-

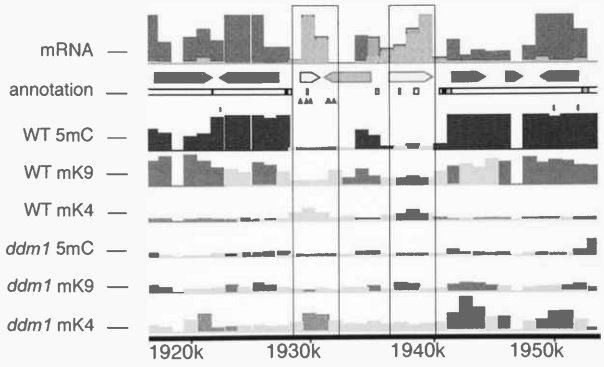

FIGURE 1 Chromatin profiling with tiling genomic microarrays. A 35-kb region from the heterochromatic knob on chromosome 4 is displayed using Genome Browser. Transcription (mRNA) is indicated for wild-type (WT) (*light gray*) and *ddm1* seedlings (*dark gray*). Annotation includes four tracks: open reading frames (ORFs), repeats, gene-trap insertions, and small RNA. ORFs are annotated as known genes (*light gray*), retrotransposons, and DNA transposons (*dark gray*). Repeats are from RepBase, gene-trap insertions are indicated by arrows, and small RNA matches by black lines. DNA methylation (5mC) and histone H3 methylation of lysine-9 (mK9) and lysine-4 (mK4) significantly above or below the average level found in euchromatic features are highlighted. Euchromatic levels of each modification are light gray. Two genes (*boxed*) are expressed, unmethylated and free of H3mK9, despite their close proximity to silent transposons.

onaute), *dcr1*$^-$ (dicer), and *rdr1*$^-$ (RNA-dependent RNA polymerase), have very similar microarray profiles: Apart from centromeric repeats, few genes are up-regulated. This indicates a limited role, if any, for microRNA (miRNA) in fission yeast, although centromeric siRNA may silence some genes near the telomere. Interestingly, coding transcripts from retrotransposable elements are only very slightly up-regulated despite the loss of H3mK9 from their long terminal repeats (LTRs). Microarray profiles were similar to those from the histone H3 lys-9 methyltransferase *clr4*$^-$, supporting this conclusion, but profiles from other silencing mutants had more widespread effects. These results are in agreement with our observations in *Arabidopsis* that transposon silencing can be maintained independently of RNAi.

Genes and Transposons Are Differentially Methylated in Plants But Not in Mammals

P. Rabinowicz, R. Martienssen [in collaboration with W.R. McCombie and S. Lowe, Cold Spring Harbor Laboratory]

We have studied the methylation of plant and animal genomes using polymerase chain reaction (PCR). Repetitive elements are methylated in both organisms, but although 85% of mammalian exons are methylated, 95% of plant exons are not. Sequencing strategies that depend on differential methylation are therefore predicted to have different outcomes in plant and mammalian genomes. Methylation filtration results in more comprehensive representation of maize genes than do expressed sequence tags (ESTs). Less than 7% of the repetitive DNA is unmethylated and thus selected in our libraries, but potentially active transposons and unmethylated organelle genomes can be identified. Gene-enriched sequences from the Maize Genomics Consortium provide almost twofold coverage of the maize gene space. Simulations using sequenced bacterial artificial chromosomes (BACs) predict that fivefold coverage of gene-rich regions and onefold coverage of BAC contigs will generate high-quality mapped sequence. By sequencing several inbred strains, unusually high levels of structural polymorphism will shed light on hybrid vigor, or heterosis (Fu and Dooner, *Proc. Natl. Acad. Sci. 99: 9573–9578.* [2002]).

Spatially Restricted miRNA Directs Leaf Polarity via *ARGONAUTE1*

C. Kidner, R. Martienssen

miRNAs are endogenous small RNA molecules that regulate the expression of matching genes via RNAi. In animals, this regulation occurs by translational control, but in plants, more perfectly matched miRNAs promote site-specific cleavage of the target. The homeodomain HD-ZIP III genes *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) are expressed on the adaxial (upper) side of leaf primordia where they direct cell fate. They are targets of the miRNAs miR165 and miR166. We have used in situ hybridization to show that miR165 accumulates on the abaxial side of the leaf primordia where it cleaves *PHB* and *PHV* mRNA. This pattern of expression changes during development, implicating the miRNA in signaling between the meristem and the leaf primordium. *argonaute1* (*ago1*) mutants are defective in RNAi and have a pleiotropic phenotype. Leaves and flowers are adaxialized and *PHB* mRNA accumulates throughout the leaf primordium, whereas miR165 is ectopically expressed in the adaxial domain. Thus, *AGO1* regulates both the accumulation and function of miRNA, perhaps accounting for enhancement of both loss-of function and gain-of-function alleles of *PHB*-related genes in *ago1*.

Mechanisms of miRNA Targeting via *ARGONAUTE1*

M. Ronemus, R. Martienssen

Double-stranded RNA can silence genes that share the same sequence via 20–24-nucleotide small RNA intermediates and RNAi. These intermediates include miRNA encoded by hairpin precursors in animals and plants. In *Arabidopsis*, the RNAi genes *ARGONAUTE1* (*AGO1*) and *DICER-LIKE1* (*DCL1*) are required for normal development, and we have examined the expression of thousands of genes in normal and mutant lines of *Arabidopsis* using microarrays. Several families of related genes have substantially altered expression in the RNAi mutants, and many of these genes match miRNA. However, not all miRNA targets are altered in abundance, perhaps reflecting the stability of cleavage products. Up-regulated genes

appear to be silenced by second-strand synthesis and degradation in addition to mRNA cleavage.

Leaf Polarity and Stem Cell Fate Are Determined by *ASYMMETRIC LEAVES1*

M. Byrne, J.M. Arroyo, R. Martienssen

Lateral organs such as leaves arise on the flanks of the shoot meristem via repression of stem cell fate, determination of founder cells, and elaboration of the incipient primordium. Development of lateral organs proceeds along proximodistal, dorsoventral, and mediolateral planes. It is thought that lamina outgrowth relies on juxtaposition of dorsal and ventral leaf domains. The Myb domain protein *ASYMMETRIC LEAVES1* (*AS1*) mediates a genetic interaction between the developing leaf and stem cells of the meristem via homeobox transcription factor genes. Modifiers of *AS1*, called *PIGGYBACK*, mediate ectopic lamina outgrowths on the adaxial leaf surface in *as1* but have little effect on leaf development on their own. We are cloning these modifiers and characterizing interactions with leaf patterning genes. *pgy* modifiers appear to confer proximodistal lamina outgrowth in response to disruption of dorsoventral patterning in *as1*. A second modifier generates a phenotype reminiscent of a palmate compound leaf, but only in the absence of *AS1*.

Genetic Control of Inflorescence Architecture in Grasses

E. Vollbrecht, R. Martienssen [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory]

ramosa1 encodes a zinc finger transcription factor that controls inflorescence architecture in maize by limiting the proliferative potential of shoot apical meristems. *ramosa2* positively regulates *ra1* transcript levels, such that increasing levels of *ra1* mRNA progressively inhibit a meristem's capacity for continued growth, thereby dictating final branch length in the inflorescence. *ra1* was targeted by selection during recent evolution, suggesting an important role in grass inflorescence architecture. In support of this, two cases of higher branch number correlate with delayed or reduced *ra1* mRNA expression. First, a quantitative trait locus (QTL) maps nearby and fails to complement *ra1*. Sequence evidence leads us to speculate that this heterochronic allele may be due to epigenetic effects. Second, in the highly branched inflorescence of *Sorghum bicolor*, diverged from maize approximately 20 mya, expression of the *ra1* ortholog (*ra1-Sb*) is delayed and reduced. A tandem duplication of *ra1-SB* and promoter rearrangement suggest a basis for its delayed RNA expression. In this region of the genome, microcolinearity between sorghum and rice is conserved with only one exception: *ra1* is absent from rice, whose inflorescence branches are long consistent with its absence.

Functional Genomics with Transposons in Maize and *Arabidopsis*

B. May, M. Vaughn, D. Roh, J. Simorowski, J.-M. Arroyo, R. Shen, A. Tang, U. Umamaheswari, R. Martienssen [in collaboration with Dick McCombie and Lincoln Stein, Cold Spring Harbor Laboratory]

We continue to serve the maize community through MTM (maize-targeted mutagenesis), an efficient system for site-selected transposon mutagenesis. MTM selects insertions in genes of interest from a library of 45,000 plants using PCR. Pedigree, knockout, sequence, phenotype, and other information are stored in a powerful interactive database (mtmDB) that enables analysis of the entire population as well as handling knockout requests. By monitoring *Mutator* activity, we conclude that more than one half of all mutations arising in this population are suppressed on losing *Mutator* activity. Our collection of *Arabidopsis* gene-trap and enhancer-trap transposon lines has grown to more than 33,000 individual insertions, about half of which have been mapped to the genome by PCR and sequencing. The resulting knockouts, along with phenotypic and expression data, are made available to the public via TRAPPER, an interactive database and ordering system. More than 200 orders were filled for maize and *Arabidopsis* lines in 2003.

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PLANT CELL SIGNALING AND GENETICS

D. Jackson S. Alabaster, U. Au, R. Chaudhury, M. Cilia, W. Deleu, L.A. Haller, J.Y. Kim, K. Lau, J. Linder, A. Mohanty, N. Satoh, J. Wang

We are interested in mechanisms of development and morphogenesis, and we use plants as a model system. Patterning of stem cells is a fundamental mechanism used by animals and plants to generate new organs and tissues throughout their development. Although some stem cell regulatory mechanisms are conserved, one patterning mechanism that is unique to plants is the intercellular transport of regulatory proteins through channels called plasmodesmata. We are studying the mechanism of transport through these elusive channels, using a combination of genetic screens and cell biology. We have also started to investigate the molecular basis of cytokinin action. This plant hormone was first isolated in the 1950s, but its mechanism of action remains elusive. Through the characterization of a novel morphological mutant, we are beginning to understand the role of cytokinin responsive genes in plant development.

A Novel Screen for KNOTTED1 Intercellular Transport

K. Lau, D. Jackson, J.Y. Kim

A novel mechanism of cell-cell communication is the direct intercellular trafficking of regulatory proteins and mRNAs through plasmodesmata (PD). We have developed a novel assay for protein trafficking by fusing the nonautonomous KNOTTED1 (KN1) homeodomain protein to *GLABROUS1* (*GL1*), a gene required for epidermal hair production. When this fusion protein is expressed in mesophyll tissues, it is able to traffic into the epidermis and complement hair production in a *glabrous1* mutant. Expression of *GL1* alone in mesophyll fails to rescue hair formation, because *GL1* itself is cell-autonomous. This system provides a powerful visual screen, and we have used it to dissect regions of KN1 required for trafficking. We found that the homeodomain of KN1 is necessary and sufficient for trafficking, and residues flanking the homeodomain significantly affect trafficking activity. We also confirmed earlier microinjection data showing KN1 is able to specifically transport its mRNA from cell to cell. Although the significance of this transport for normal development is not known, it provides a potential for powerful nonautonomous control of plant development by long-range signaling, e.g., in signaling by mRNAs that are transported in the circulatory phloem stream. We are also using the *GL1* system to screen for mutants impaired in cell-to-cell trafficking of KN1, by screening for lines in which hair formation is no longer complemented. Such mutants will help dissect the mechanism of KN1 transport.

Isolation and Characterization of Plasmodesmata Mutants

M. Cilia

Using a genetic approach to understand PD structure and function, we have isolated a number of mutants that affect the intercellular movement of the green fluorescent protein (GFP). The GFP is small enough to move nonselectively through PDs in immature "sink" tissues, such as in the root meristem. We used this property to screen for mutants that restrict GFP movement, and which presumably have a decreased PD size-exclusion limit. Interestingly, some of the mutants have developmental phenotypes, supporting the idea that plasmodesmal transport is important in developmental regulation. For example, one mutant, called "5-12," has a complete loss of GFP movement out of the phloem (Fig. 1A). This mutant is seedling lethal, supporting the idea that PD transport is essential for normal development. Other mutants define tissue boundaries for PD transport, supporting a model of developmental specificity in PD transport. We are currently mapping the mutants with the aim of isolating the corresponding genes.

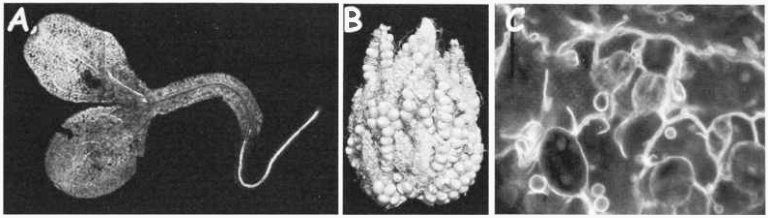

FIGURE 1 Plasmodesmata mutant (*A*) shows an early seedling lethal phenotype. In *B*, the *ramosa3* mutant of maize, with abnormally branched ears. Localization of a fluorescent protein reference gene reporter to the vacuolar membrane (*C*).

Fine Mapping of the *ramosa3* Locus of Maize

N. Satoh

ramosa3 (*ra3*) is a classical morphological mutant of maize in which the female inflorescence becomes abnormally branched, resembling the male inflorescence structure (Fig. 1B). In order to isolate the *RA3* gene, we embarked on a chromosome walk using a large F2 mapping population. We have narrowed the *RA3* locus to a region smaller than one BAC (bacterial artificial chromosome), which is currently being sequenced (in collaboration with Hajime Sakai, Dupont). The syntenic region in rice contains only hypothetical genes, suggesting that RA3 may encode a gene of previously uncharacterized function. Following BAC sequencing, we plan to identify the *RA3* gene by characterization of several independent mutant alleles that we isolated in targeted screens.

An *Arabidopsis* "Localizome" Project

J. Wang, S. Alabaster, A. Mohanty

Approximately one third of all plant genes identified in sequencing projects have no known function. Some are plant-specific, whereas others match genes in other organisms where their function also is unknown. As a first step in understanding their function, we developed a high-throughput procedure to tag full-length genes in their native genomic context using fluorescent reporters. These constructs are subsequently transformed into *Arabidopsis*, and the transgenic progeny are analyzed for protein localization. We propose to characterize several thousand genes of unknown function and to integrate our results with other proteomics and mutational approaches to understand the function of all plant genes. An example of a control tagged protein, which localizes to the vacuolar membrane, is shown in Figure 1C.

Characterization of the *ABPHYL1* Phyllotaxy Regulator

J. Wang, D. Jackson

Plants initiate lateral organs, such as leaves, in regular geometric arrangements, as seen, for example, in the spiral patterns of sunflower heads. The way in which these patterns are established in presently unknown. We have characterized a phyllotaxy mutant of maize called *abphyl1*. In this mutant, leaves are initiated in opposite symmetry, a pattern never seen in maize or other grasses. We isolated *abph1* using transposon tagging, and it encodes a cytokinin-inducible response regulator. Recent studies implicate auxin, another plant hormone, in maintenance of phyllotactic patterns. Our results support a model whereby the initial pattern is set up by localized cytokinin signals, and we have shown that exogenously applied cytokinin can change the pattern of *ABPH1* mRNA accumulation in the shoot meristem.

Further details and updates can be found at http://jacksonlab.cshl.edu/index.html.

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PLANT DEVELOPMENTAL GENETICS

M. Timmermans G. Collins M. Juarez T. Phelps-Durr J. Thomas

ANALYSES OF GENES INVOLVED IN MERISTEM FUNCTION AND LEAF INITIATION

This work was done in collaboration with M. Scanlon (University of Georgia, Athens); P. Schnable (Iowa State University, Ames); B. Buckner and D. Janick-Buckner (Truman State University, Kirksvile, Missouri).

Plants have the unique property to be able to initiate new organs, such as leaves and flowers, throughout their lifetime, which frequently extends over many years. The growing tips of plants are called meristems. The vegetative shoot apical meristem (SAM) exhibits distinctive histological and functional domains. For instance, the SAM comprises distinct epidermal and subepidermal cell layers and includes a small population of indeterminate stem cells as well as more determined daughter cells from which lateral organs arise. Traditional genetic analyses have identified several genes that are required for meristem function or for the development of lateral organs from the SAM. Such studies have also shown the importance of this meristematic organization for normal plant development.

To identify novel and potentially redundant or essential genes that function in discrete domains of the SAM or developing leaf primordia, we are using a technique called laser-capture microdissection (LCM), which allows the isolation of transcripts from specific cell types within a particular tissue or organ. For use in LCM, target tissues are fixed, sectioned, and immobilized onto microscope slides. Cells of interest are dissected and separated from the rest of the tissue using a laser beam. RNA is then extracted from the captured cells and used in microarray analyses to compare global gene expression patterns between different cell populations.

We are currently optimizing the LCM technique, which was originally designed for the dissection of animal cells, for use on the small-cell-wall-encapsulated cells within the plant SAM. We are using maize as our experimental system because of the relatively large size of its vegetative meristem when compared to, for instance, *Arabidopsis*. We have found that fixed and paraffin-embedded tissues can be used as efficiently as frozen tissues for LCM. RNA collected from as few as a thousand cells was sufficient for the preparation of cDNA for use in microarray analyses of gene expression. Although maize is a particularly tractable model system for LCM of meristems, maize has the disadvantage that its genome project is still at an early stage. To identify genes that should be represented on the long-oligo microarrays, we have also initiated a "small" expressed sequence tag (EST) project. The goal is to generate approximately 35,000 3′ ESTs from meristem-enriched cDNA libraries.

We intend to compare global expression patterns between approximately 20 different functional meristematic and leaf primordia domains, or experimental conditions. These include the following comparisons: stem cells versus more determined daughter cells, meristematic cells versus leaf initials, cells in the leaf's upper/adaxial surface versus its lower/abaxial surface, epidermal versus subepidermal cells, and between apices of normal and a variety of developmental mutants.

Genes that exhibit differential expression in a variety of developmental domains, mutations, and growth conditions will be identified and verified by real-time reverse transcriptase–polymerase chain reaction (RT-PCR) and in situ hybridization analyses. Even though this project is still in its infancy, it will soon provide a novel insight into meristem function and organogenesis.

REPRESSION OF STEM CELL FATE DURING ORGAN DEVELOPMENT

This work was done with contributions from Josh Egan and Jason Pellegrino (Manhasset High School) and Arooba Alam (Locust Valley High School).

The *knox* (*knotted1*-like homeob*ox*) gene family is known to be required for indeterminate growth, i.e.,

stem cell function, in a wide variety of plant species. Mutations in several *knox* genes affect meristem initiation and/or maintenance. Significantly, in simple-leaved plants such as maize and *Arabidopsis*, KNOX proteins accumulate in the indeterminate cells of the SAM but are excluded from determinate leaf founder cells. Moreover, in these species, down-regulation of *knox* genes is essential for normal leaf development. Misexpression of *knox* genes within developing simple leaves leads to cell division and differentiation defects, which can be compared to tumor formation in animals. The *rough sheath2* (*rs2*) gene from maize and the *ASYMMETRIC LEAVES1* (*AS1*) gene from *Arabidopsis* encode orthologous MYB-domain proteins that act as negative regulators of *knox* gene expression. On the basis of our previous expression and genetic analyses, we proposed that *rs2* acts as an epigenetic regulator to keep *knox* genes in an "off" state in developing lateral organs, thus preventing differentiating cells from reverting into indeterminate stem cells.

We have shown that RS2 is part of a large protein complex that likely includes protein phosphatase 2A regulatory subunits A and B′, a zinc-finger transcription factor, the homolog of ASYMMETRIC LEAVES2 (AS2) from *Arabidopsis*, a highly conserved DEAD-box helicase, histone 2B (H2B), and a known nucleosome assembly protein. By transiently expressing chimeric proteins between these RS2-interacting proteins (RS2-IPs) and green fluorescent protein (GFP) in leaf tissue, we have shown that all seven proteins are targeted to the nucleus, consistent with a role in transcriptional regulation. The fusion protein between GFP and the DEAD-box helicase colocalized with chromosomes in dividing cells, suggesting that it is associated with chromatin. Interestingly, the nucleosome assembly protein was found to accumulate within specific subnuclear structures; however, the nature and function of these structures remains to be determined.

The presence of a nucleosome assembly protein and a DEAD-box helicase in the RS2 complex, as well as its potential interaction with H2B and protein phosphatase 2A, suggests that this complex may indeed maintain *knox* genes silenced by altering the organization of chromatin at these loci.

Actively transcribed genes usually have a more open chromatin structure, i.e., a lower nucleosome density, due to the presence of specific modifications at the tails of the different histone proteins that make up the nucleosome. For instance, phosphorylation is one of the histone tail modifications associated with actively transcribed genes. We are currently analyzing the binding sites of the RS2 complex at the *knox* loci and are testing whether chromatin organization or histone modifications at these loci are affected in the *rs2* mutant.

Last year, we also isolated *Arabidopsis* homologs for most of the RS2-IPs and found that the AS1 complex is very similar to the maize RS2 complex. One striking difference between the two complexes is that the *Arabidopsis* homolog of the zinc-finger transcription factor does not appear to interact with AS1. We are using the reverse genetic resources available in *Arabidopsis*, to determine the roles of the AS1/RS2-IPs in plant development, particularly in stem cell function. Loss-of-function mutations in *AS2* are known to affect *knox* gene silencing during leaf development, but our results indicate that AS2 acts in the same complex as AS1/RS2.

We have isolated mutations in the *Arabidopsis* orthologs of the DEAD-box helicase and the nucleosome assembly protein. Loss-of-function mutants in the DEAD-box helicase are indistinguishable from wild type.

In contrast, loss of the nucleosome assembly protein causes embryo lethality, indicating that it has an essential role. However, we have generated transgenic plants that have reduced levels of the nucleosome assembly protein using an RNA-interference-like approach. These transgenic plants have defects in leaf and floral development that resemble the defects observed in the *as1* and *as2* mutants. The sepals, petals, and stamens of the flower are reduced in size, such that the carpel is prematurely exposed. Leaves of these transgenic plants are asymmetrically lobed and like *as1* and *as2*, they misexpress three of the four *Arabidopsis knox* genes. *AS2* expression is unaffected in these mutants, but *AS1* transcript levels are increased, indicating that the defects in these transgenic plants do not result from loss of *AS1* or *AS2*. Double-mutant analysis revealed that reduced levels of the nucleosome assembly factor enhance the *as1* and *as2* mutant defects. Such double mutants develop extremely lobed leaves.

Taken together, our observations suggest that AS1/RS2 and AS2 assemble into a protein complex which targets a nucleosome assembly factor and possibly other chromatin remodeling proteins to the *knox* loci in organ founder cells. As a result, stem cell fate remains repressed in determinate, differentiating cells of developing lateral organs.

ROLE OF THE RS2/AS1/PHAN COMPLEX IN COMPOUND LEAF DEVELOPMENT

This work was done in collaboration with M. Kim and N. Sinha (University of California, Davies) and S. McCormick (Plant Gene Expression Center, Albany, California).

Many plants species develop compound rather than simple leaves. Compound leaves comprise multiple units of blade tissue called leaflets and can be pinnate, with leaflets arranged in succession on the rachis ("stem" of the leaf), or palmate, with leaflets clustered together at the leaf tip. Palmate compound leaves are divided into two groups. Peltate palmate leaves develop leaflets around the whole circumference of the rachis, whereas nonpeltately palmate leaves form an incomplete circle of leaflets. The mechanisms that generate these various compound leaf forms are largely unknown, but compound leaves differ from simple leaves in that they remain partially indeterminate and express *knox* genes. Nonetheless, the genomes of many compound-leafed species include one or more closely related homologs of *rs2* and *AS1*.

In tomato, which develops pinnately compound leaves, this homolog, *LePHAN*, was found to be expressed in the SAM and on the adaxial/upper leaf surface of developing leaf primordia. This expression pattern thus partially overlaps with the *knox* expression domain, suggesting that *LePHAN* has a different role during development. To determine this role, our collaborators used antisense technology to reduce the levels of *LePHAN* expression. They obtained transgenic plants that developed palmate compound, simple, or radially symmetric abaxial leaves, and these phenotypes were correlated with a progressive reduction in *LePHAN* transcript levels. This suggested that RS2/AS1/PHAN in tomato is required for the adaxial/upper cell fate consistent with the *LePHAN* expression pattern and for compound leaf shape.

Analysis of pinnate, non-peltately palmate, and peltately palmate leaves from several hundred species from a variety of taxa revealed that the rachis of pinnate leaves contained adaxial/abaxial asymmetry. Similarly, the rachis of non-peltately palmate leaves had adaxial/abaxial polarity, and no leaflets were found to develop in the adaxial domain.

In contrast, the petioles of peltately palmate leaves were abaxial and had radial symmetry. This suggested that the absence of the adaxial domain in the proximal region of the rachis or petiole is important for generating peltately palmate compound leaves. To test whether RS2/AS1/PHAN homologs may have had a role in the evolution of compound leaf forms, members of this gene family were cloned and their expression domains determined using an affinity-purified polyclonal antibody that we had generated against RS2. Sequence analysis revealed that alterations in the coding region of PHAN did not have a role in the evolution of compound leaf morphology. However, we found that variation in the pattern of PHAN expression may have had a crucial role in this evolutionary process. PHAN was expressed along the entire adaxial face of leaf primordia in pinnate compound-leafed species. In contrast, we observed PHAN expression only at the distal tip of leaf primordia in species with peltately palmate leaves.

Interestingly, the PHAN expression pattern in non-peltately palmate leaf primordia resembled that in pinnate leaves. Using scanning electron microscopy, we showed that leaflet development at early stages was almost identical in pinnate and non-peltately palmate compound leaves and that final leaf morphology was determined by secondary morphogenesis later in leaf development. This suggested that non-peltately palmate leaves are a developmental variation of pinnate compound leaves and are morphogenetically distinct from peltately palmate leaves. These data also indicate that RS2/AS1/PHAN proteins have distinct functions in simple and compound-leafed species. In the latter, RS2/AS1/PHAN specifies the adaxial domain.

The boundary between adaxial and abaxial domains seems to be required for leaflet formation, such that in peltately palmate compound leaves, leaflets arise in a whorl in the distal region of the leaf primordium because an adaxial domain is present only in this region. Moreover, the control of leaf morphology by regulating PHAN expression seems to be reused over time, suggesting that there may be limited ways to alter compound leaf morphogenesis.

ADAXIAL/ABAXIAL PATTERNING OF LATERAL ORGANS IN MAIZE

This work was done with contributions from Jonathan Kui (Watson School of Biological Sciences, CSHL, rotation student) and Bradley Heller (Princeton University, New Jersey).

Recruitment of leaf founder cells from the SAM coincides with programs of development and differentiation along three axes, comprising the mediolateral

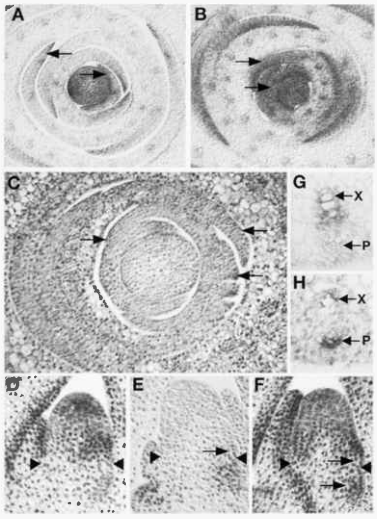

FIGURE 1 Complementary *hd-zipIII* and miRNA166 expression. (*A*) Transverse section through a wild-type apex showing *rld1* expression in the SAM, vasculature, and on the adaxial side of leaf primordia (arrows). (*B*) *rld1* misexpression on the abaxial side of *Rld1-O* leaf primordia (*arrow*). (*C*) miRNA166 expression in leaf primordia but not in the SAM. Arrows mark regions with just abaxial miRNA166 expression. (*D,E*) Longitudinal sections through wild-type apices showing *rld1* expression (*D*) at the site of leaf initiation and miRNA166 expression (*E*) on the abaxial side (arrow) and below the incipient leaf. Arrowheads mark the top of the P1 disk of insertion. (*F*) *rld1* misexpression near the incipient leaf in *Rld1-O* (*arrows*). *phb* (*G*) or miRNA166 (*H*) expression in wild-type vascular bundles. (*X*) Xylem; (*P*) phloem.

(margin to margin), proximodistal (base to tip), and adaxial/ abaxial (upper to lower) axes of the leaf primordium. We are using forward and reverse genetic approaches to analyze the genetic pathways leading to adaxial/ abaxial polarity in lateral organs in maize. The *Rolled leaf1-Original* (*Rld1-O*) mutant is semidominant and is characterized by an upward curling of the leaf blade due to adaxialization or partial reversal of leaf polarity. We recently cloned the *rld1* gene. It encodes a member of the class III homeodomain-leucine zipper (HD-ZIP III) family of proteins, which are known to specify adaxial fate in *Arabidopsis*. We found that *rld1* is expressed in the SAM, vasculature, and, consistent with a role for *rld1* in adaxial cell-fate specification, nondetermined cells on the adaxial side of leaf primordia (Fig. 1A,D). The meristematic expression pattern of *rld1* was unaffected in *Rld1-O*. However, we observed that *rld1* is misexpressed on the abaxial side of *Rld1-O* leaf primordia and persists in a broader domain during *Rld1-O* primordium development (Fig. 1B,F).

Transcripts of the *Arabidopsis HD-ZIP III* genes contain a complementary site for microRNA165 (miRNA165) and miRNA166, which can direct their cleavage in vitro. We found that this miRNA165/166 complementary site is conserved in the maize *rld1* gene and that all four dominant *Rld1* alleles result from a single nucleotide change in this site. This, together with the misexpression of *rld1* in the *Rld1-O* mutant, suggests that miRNAs may mediate the post-transcriptional repression of *rld1* on the abaxial side of leaf primordia. In other words, miRNA165/166 may specify abaxial cell fate by restricting expression of *rld1* and other *hd-zipIII* genes to the adaxial side.

miRNAs are processed from larger imperfect hairpin precursors (pre-miRNA) by the double-stranded ribonuclease *DICER-LIKE1*. *Arabidopsis* contains two potential pre-miRNA165 (*MIR165*) and seven *MIR166* loci. The rice genomic sequence includes at least six loci with the potential to generate miRNA166 homologs.

We identified several sequence motifs outside the predicted hairpin structures that are conserved between *Arabidopsis MIR166* family members. Some of these motifs were also conserved in two rice *MIR166* genes, suggesting that they constitute part of the pre-miRNA transcript or contain elements important for miRNA166 regulation which may also be conserved in maize. Using degenerate primers derived from these conserved sequence motifs, we cloned fragments from four maize *mir166* loci.

These fragments were used as probes in in situ hybridization experiments to examine whether miRNAs can indeed establish patterns of tissue differentiation during development. Consistent with the relatively low abundance of precursor transcripts, we only detected hybridization using probes directed against the miRNA. We did not detect any miRNA166 expression in the SAM, suggesting that the meristematic *rld1* expression domain is controlled at the transcriptional level (Fig. 1C,E). However, *rld1* and miRNA166 exhibited complementary expression patterns in developing leaf primordia consistent with a

role for miRNA166 in the spatial regulation of *rld1* transcripts.

miRNA166 was expressed immediately below the incipient primordium and formed a gradient into the abaxial side of the incipient leaf (Fig. 1E). In older leaf primordia, miRNA166 accumulated in a progressively broader domain extending laterally and adaxially (Fig. 1C). This dynamic expression pattern and the possible gradient of miRNA166 expression in leaf primordia are reminiscent of a movable signal, suggesting that expression of the miRNA166 precursor could be under such control or, alternatively, that miRNA166 can move between cells.

hd-zipIII genes function also in adaxial/abaxial patterning of vascular bundles. We found that vascular *hd-zipIII* expression is indeed limited to the adaxial pro-xylem cells (Fig. 1G), whereas miRNA166 accumulates in the abaxial phloem tissue (Fig. 1H). This suggests that specification of adaxial/abaxial polarity during leaf and vascular development is in part governed by the same mechanism. Moreover, the accumulation of miRNA166 in phloem led us to propose that miRNA166 may move from the site of *mir166* expression. miRNA165/166 thus constitutes an important highly conserved polarizing signal that may emanate from a signaling center below the incipient leaf. Early surgical experiments suggested that specification of adaxial cell fate requires a meristem-borne signal, as separation of incipient primordia from the SAM by incision causes formation of radially symmetric abaxialized leaves. In addition to the DNA-binding domains, HD-ZIP III proteins contain a START-like lipid-sterol-binding-like domain, suggesting that they may specify adaxial fate by conveying this meristem-borne signal. Thus, *rld1* and other *hd-zipIII* genes may specify adaxial/abaxial polarity by perceiving and integrating two positional signals: the meristem-borne signal and the miRNA signal emanating from below the incipient leaf.

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COLD SPRING HARBOR LABORATORY FELLOWS

In 1986, Cold Spring Harbor Laboratory began a Fellows program to encourage independent research by outstanding young scientists who, during their graduate studies, displayed exceptional promise of becoming leading scientists of the future. The purpose of this program is to provide an opportunity for these young scientists to work independently at the Laboratory for a period of up to three years on proj-ects of their own choosing. Fellows are provided with a salary, research support, and technical assistance so that they can accomplish their goals free of distraction. The interaction among research groups at the Laboratory and the program of courses and meetings on diverse topics in biology contribute to a research environment that is ideal for innovative science by these Fellows.

Previous Cold Spring Harbor Laboratory Fellows Adrian Krainer (1987) and Scott Lowe (1995) are currently members of the faculty at the Laboratory. After 9 years at the Laboratory, Carol Greider (1988) left to join the Department of Molecular Biology and Genetics at Johns Hopkins University School of Medicine. Eric Richards (1989) is currently in the Department of Biology at Washington University. After finishing his fellowship, David Barford (1991) returned to the Laboratory of Molecular Biophysics at Oxford University. Ueli Grossniklaus (1994) was a member of our faculty before leaving to join the Friedrich Miescher Institut in Basel, Switzerland in 1998. Marja Timmermans, who joined us from Yale in 1998, ended her fellowship in June of 2002, when she became an assistant professor at the Laboratory.

The two current CSHL Fellows, Gilbert (Lee) Henry and Terence Strick, joined the Laboratory in 2000. Their reports are listed below. Lee joined us from Doug Melton's laboratory at Harvard University where he earned his Ph.D. for studies on *Xenopus* development. Lee is studying taste bud development and the role of innervation in this process. Terence joined us after earning his Ph.D. in molecular and cellular biology at École Normale Supérieure in Paris with David Bensimon and Vincent Croquette. Terence is using single-molecule biophysics to study the mechanical response of DNA to stretching and twisting by enzymes that alter DNA topology, thus elucidating the properties of these enzymes.

L. Henry
T. Strick

Structural and Functional Studies of the Vertebrate Taste Bud

L. Henry, Y. Zhu, M. Siddiqui

The goal of our research is to understand the molecular mechanisms required for the formation of taste buds during embryogenesis and the maintenance of their structure and function in the adult. The sensory cells of gustation are housed within ovoid structures called taste buds which are embedded in the epithelium of the tongue, and to a lesser extent the epithelium of the palate and upper pharynx. Consisting of 70–100 cells, the vertebrate taste bud is a highly dynamic structure that possesses both epithelial and neuronal qualities. Like all other sensory cell types, the taste receptor cell membrane depolarizes in the presence of a suitable stimulus. Similar to the epithelial cells that line the intestine and other areas of the gut, cells within the bud turn over at a rapid rate (~8–10 days in rodents). Unlike the olfactory system, where the axons of newly formed receptor cells project for some distance back toward and synapse on to neurons in the glomeruli of the olfactory bulb, newly formed taste receptor cells lack classical projections and synapse on to sensory afferents associated with the taste bud.

Mammalian taste buds are embedded in epithelial specializations called papillae. On the surface of the tongue, three classes of papillae are easily distinguished. Toward the back of the tongue, there is a centrally located circumvallate papilla, two groups of foliate papillae are found to the lateral extremes of the

posterior tongue, and scattered over the anterior tongue are reddish colored fungiform papillae. The papillae form late during embryogenesis in the mouse (E13–E16) as the tongue begins to bud away from the pharynx. Soon after their formation, sensory afferents enter the papillae, and approximately eight to ten days after birth (P8–P10), taste buds form. In rodents, there is a single bud per fungiform papillae, tens of buds within the two foliate papillae, and hundreds of buds are found in the single circumvallate.

An intimate relationship between both the developing papillae and the mature taste bud with innervating sensory afferents has been established through a number of denervation studies. Papilla formation does not require innervation; however, the maintenance of these structures does in some way require contact with sensory afferents. Similarly, in adult animals, denervation of the tongue leads to the loss of taste buds. We are currently trying to understand the molecular basis for this neuroepithelial interaction. However, before regulatory mechanisms can be understood, it is first necessary to understand exactly what is being regulated. Toward this end, a primary aim of our current work is to understand, in molecular detail, how many different types of cells reside in the developing papilla and adult taste bud. What, if any, lineage relationships exist among these cell types? Is the distribution of the different cell types that reside in these structures spatially patterned?

DEVELOPMENT OF A QUANTITATIVE METHOD FOR THE ANALYSIS OF mRNA IN SINGLE CELLS?

A prerequisite for much of our work is the development of a quantitative method for the analysis of mRNA expression in single or small numbers of cells. A typical eukaryotic cell contains picogram amounts of mRNA, whereas the vast majority of the analytical procedures that we use to analyze gene expression require microgram amounts of material. Thus, the mRNA expressed in a single cell must be amplified before it can be studied. We have developed a novel scheme for performing such amplifications. In this technique, mRNA is bound to a magnetic bead, covalently coupled cDNA is produced, and after a series of molecular manipulations, the cDNA is released and amplified by either polymerase chain reaction (PCR) or in vitro transcription. The advantage of performing such manipulations in the solid phase is that precipitation and chromatographic steps are not required between manipulations, which allows the user a great deal of flexibility in modifying the cDNA so that it can be amplified. We have successfully amplified mRNA from single adult taste receptor cells and taste buds. Additionally, we are using microarray technology to assess the quantitative abilities of our procedure.

These experiments simply involve comparing the expression profiles of diluted and amplified material versus undiluted material. An additional concern relates to the ratio of signal and noise observed in our amplifications. For example, it is not uncommon when amplifying cDNA from single cells to find material in the amplified product that is not derived from the cell of interest. We have established the source of this noise and have devised a method to eliminate it from the amplified product. We expect and are currently testing the idea that the sensitivity of our assay will be aided by a reduction in amplification noise.

HOW DIVERSE ARE BOTH THE MATURE RECEPTOR CELL POPULATION AND THE PROGENITOR POOL FROM WHICH IT IS DERIVED?

Our goal is to develop a molecular fingerprint for each of the different cell types that reside in the adult taste bud. By fingerprint, we essentially mean a transcriptional profile. From the perspective of cell division, there are two types of cells within the adult taste bud: mitotically active progenitor cells at the base of the bud and differentiated quiescent cells at the apex. Mature taste receptor cells fall into the later category. In addition, the differentiated cells of the taste bud are highly elongated as compared to the progenitors and can release neurotransmitter onto the sensory afferent network with which they form multiple synapses. These properties allow basal cells to be distinguished from their mature, apically located, daughter cells by the presence of mRNA transcripts that encode proteins that support the aforementioned processes. For example, there are a small number of genes known that are only expressed in dividing cells, and the production of certain neurotransmitters requires biosynthetic enzymes that are only expressed by the cells that make and release the transmitter. Altogether, we have isolated just over 100 cDNA clones that can be used to presort cells. Each of these clones has been spotted onto a low-density microarray which is hybridized to amplified and labeled cDNA derived from single cells. This pre-sorting hybridization allows progenitor and mature cells to be distinguished, as well as bitter-, sweet-, sour-, and salt-responsive cells.

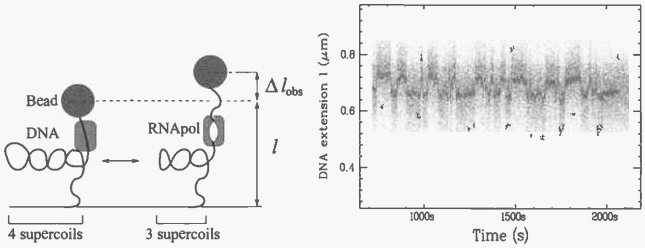


FIGURE 2 Observation of promoter melting by a single RNA polymerase. (*Left*) Sketch of the experiment. DNA containing a single bacterial promoter site is negatively supercoiled, causing it to form supercoiled loops and reducing the end-to-end extension. When RNA polymerase unwinds the promoter site, topological coupling in the DNA causes a supercoil to disappear and the extension increases. When the promoter site reanneals, the supercoil reappears and the extension decreases. (*Right*) In the presence of RNA polymerase, the DNA extension does indeed fluctuate between a low-extension/closed promoter state and a high-extension/open promoter state.

studies of the interactions between RNA polymerase and DNA (Revyakin et al. 2003, 2004). We have been particularly interested in how DNA supercoiling affects the process of transcription initiation, a fundamental step in the regulation of gene expression. A sketch of single-molecule detection of promoter melting is provided Figure 2.

For these experiments, we prepared a 4-kb DNA molecule containing a single bacterial promoter site. Upon addition of bacterial RNA polymerase holoenzyme, we are able to observe large-scale fluctuations in the DNA end-to-end extension. These fluctuations are consistent with promoter unwinding/rewinding (see Fig. 2). Since these fluctuations are not observed if the DNA does not contain the promoter site, they must be due to interactions between an RNA polymerase and the promoter site. By measuring the amplitude of these fluctuations, we can estimate the extent of DNA untwisting which takes place during promoter opening, as well as observe bending of the promoter DNA by the RNA polymerase.

Since these observations are performed in real-time, we are able to directly determine the kinetics of formation and destruction of the RNA polymerase/unwound promoter complex. We find that the time-scale of the RNA polymerase/DNA intearction is extremely sensitive to the degree of supercoiling of the nanomanipulated DNA. Since the kinetics of a reaction reflect its energetic cost, we determined that it is the torque generated by DNA supercoiling which affects the rate of transcription initiation. The RNA polymerase/promoter complex literally behaves like a torque sensor. Since DNA supercoiling is actively regulated in vivo by the topoisomerase enzymes, this suggests that DNA topology constitutes a means for the cell to regulate gene expression.

This work shows that single-molecule DNA nanomanipulation is a robust methodology for the study of protein-DNA interactions and, in particular, those involved in transcription initiation. In principle, processes such as the initiation of DNA replication could also be studied using this technique. In the future, we will combine this nanomanipulation technique with single-molecule fluorescence detection in order to measure protein conformational changes in parallel with our measurements on DNA topology.

DNA Compaction by SMC Proteins

T. Kawaguchi, M. Hirano, T. Hirano, T. Strick

A second project that we have been pursuing involves the study of DNA compaction by the SMC (structural maintenance of chromosome) proteins such as *Bacillus subtilis* SMC and *Xenopus laevis* condensin complex.

Using DNA nanomanipulation, we are able to observe large-scale, rapid and yet reversible condensation of the DNA upon addition of purified *X. laevis* condensin and ATP. Control experiments using ATP analogs and condensin purified from different phases of the cell cycle show that both mitotic phos-

HOW MANY TYPES OF CELLS ARE CONTAINED WITHIN THE DEVELOPING PAPILLAE?

To understand how papillae are formed and innervated, it is imperative that we first ask what are papillae, at the molecular level. The papillae of the tongue are very similar in structure to the various ectodermal placodes that cover the epidermis and give rise to hair, feathers, and teeth. The secreted signaling factor sonic hedgehog (*shh*) is expressed in cells that will form these structures and that expression is maintained after the papilla or placode has formed. We plan to exploit this finding by using the *shh* locus to ectopically express GFP in developing papillae. Bacterial artificial chromosome (BAC) clones carrying the *shh* gene have been isolated, and using a recently described recombination technique, a GFP cassette has been inserted into this locus. Transgenic mice will be generated using the modified BACs. Single GFP-positive cells from the lingual epithelium of E12–E17 embryos will be isolated and transcriptionally profiled using a single-cell cDNA synthesis method (see below). Profiles will be obtained by screening microarrays and direct sequencing of cDNAs. From this work, we hope to determine the molecular diversity of the papillae during its formation and innervation. Once we can "fingerprint" the cells of the papillae in this manner, it should be possible to analyze the mechanisms that regulate this diversity, in particular, the contribution of innervation to the development of papillae.

PUBLICATION

Henry G.L., Zito K., and Dubnau J. 2003. Chipping away at brain function: Mining for insights with microarrays. *Curr. Opin. Neurobiol.* **13:** 570–576.

Protein-DNA Interactions

T.R. Strick

During the past year, we have made significant progress in our efforts to understand the physical basis of protein-DNA interactions. Because the cell continuously regulates the level of mechanical strain to which its DNA is subjected (e.g., via supercoiling), we have been investigating the ways in which this mechanical strain is used to regulate protein-DNA interactions. In particular, we have been using single-molecule nanomanipulation techniques to modify and study the structure and topology of DNA in processes such as gene transcription or the packaging of DNA into mitotic chromosomes.

The single-molecule experiment we have implemented is depicted Figure 1. A 4-kb linear DNA molecule (~1 μm long) is shown anchored at one end to a glass surface and at the other end to a 1-μm-diameter magnetic bead. The field generated by magnets located above the sample is used to pull on and rotate the magnetic bead, thus stretching and twisting the tethered DNA. The stretching force applied to the DNA via the bead is calibrated as a function of magnet position (the closer the magnets, the higher the force). The torsion imparted to the DNA is exactly equal to the number of clockwise or counterclockwise rotations performed by the magnets and imposed on the bead. The double helix is thus quantitatively and reversibly supercoiled while held under tension. By determining the position of the magnetic bead, one measures and calibrates the end-to-end extension of the DNA molecule, and thus its mechanical response to stretching and twisting.

Single-molecule Studies of Transcription Initiation

A. Revyakin, R.H. Ebright, T. Strick

During the past year, we have made progress in using single-DNA nanomanipulation to perform real-time

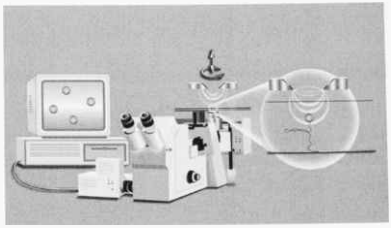


FIGURE 1 Depiction of the experimental setup. (*Inset*) DNA is tethered at one end to the wall of a glass capillary tube and at the other to a magnetic bead. (*Overview*) The capillary tube is mounted on an inverted microscope whose focus is controlled by a computer. The computer also controls the displacements (translation and rotation) of the magnets used to manipulate the bead. A CCD camera connected to the microscope relays video images of the magnetic bead to the computer. The computer extracts from these images the mean position and the Brownian fluctuations of the bead, which can be used to determine the DNA's end-to-end extension *l* which results from an applied stretching force *F* and a supercoiling of *n* turns.

phorylation of condensin and ATP hydrolysis are required for enzyme activity. Control experiments performed in the absence of ATP show that under these conditions, the condensin complex binds reversibly to DNA but does not cause its condensation. At low protein concentration, the incremental compaction of DNA can be observed as large (~60 nm) and discrete changes in DNA extension and presumably corresponds to the association and dissociation of a single condensin complex from DNA. Surprisingly, DNA supercoiling does not appear to alter the length-scale of DNA condensation events. This suggests that condensin compacts DNA by altering global DNA topology, rather than local DNA topology, possibly by driving the formation of large-scale loops along the DNA.

Torque-induced Structural Transitions in DNA

T. Strick, R. Sachidanandam

These experiments aim to better understand the physical properties of DNA. We have observed that an unwinding torque applied to DNA induces transient alterations of the molecule's secondary structure. Analysis of these conformational changes could help us understand how DNA supercoiling affects processes such as the initiation of DNA replication or the termination of transcription. We are currently working to determine the way in which the DNA sequence affects these structural transitions, and expect in this way to learn more about the kinetics and energetics of formation of alternative DNA structures such as cruciforms and denaturation bubbles.

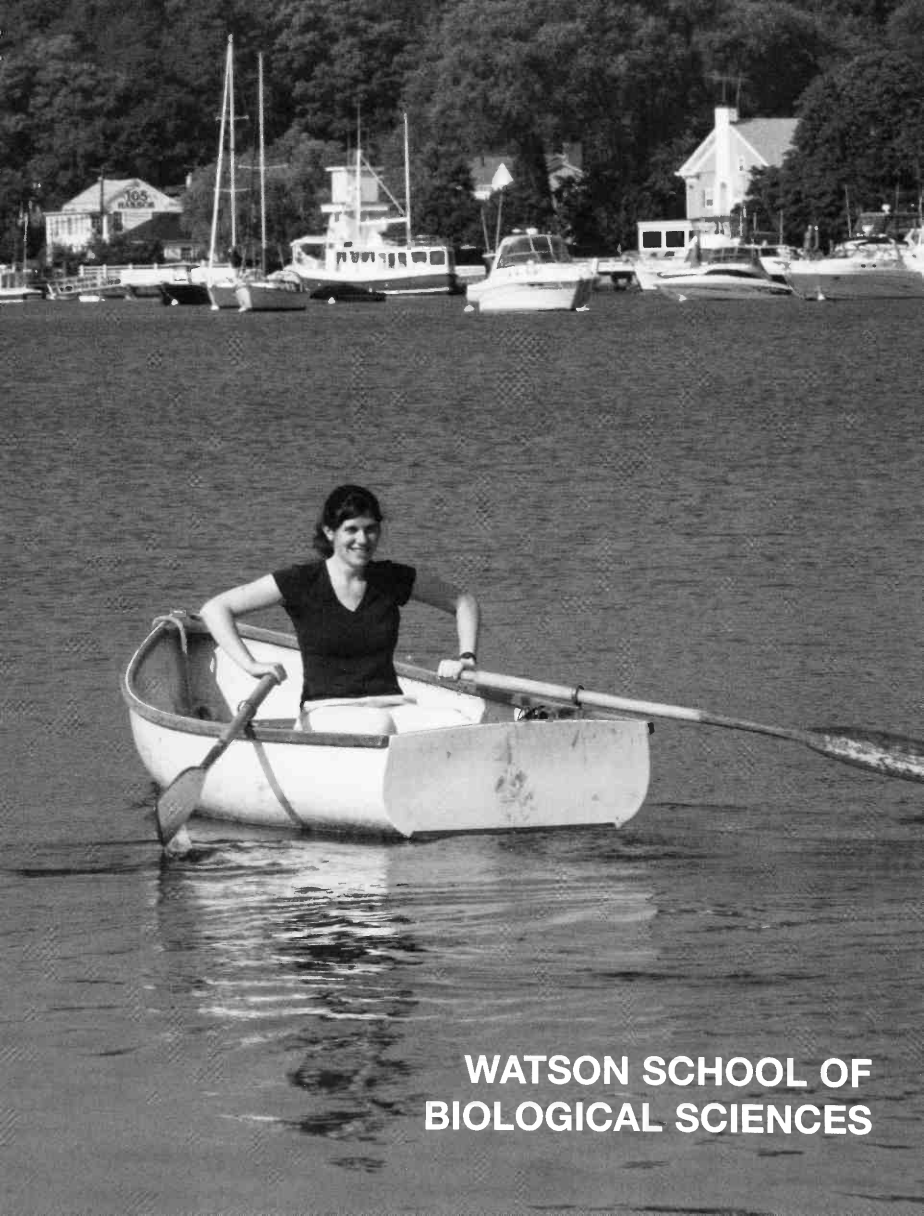
PUBLICATIONS

Revyakin A., Allemand J.-F., Croquette V., Ebright R.H., and Strick T.R. 2003. Single-molecule DNA nanomanipulation: Detection of promoter-unwinding events by RNA polymerase. *Methods Enzymol.* **370:** 577–598.

In Press

Revyakin A., Ebright R.H., and Strick T.R. 2004. Promoter unwinding and promoter clearance: Detection by single-molecule DNA nanomanipulation. *Proc. Natl. Acad. Sci.* (in press).

AUTHOR INDEX



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WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

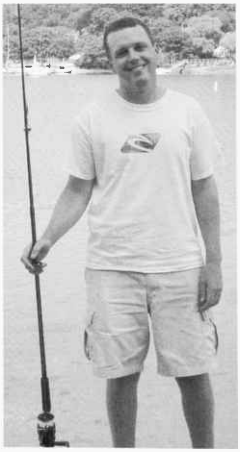
May 13, 2003 saw the Watson School achieve perhaps the most important landmark of its young existence. On that date, Amy Caudy of the first entering class in the fall 1999 became the first Watson School student to defend her doctoral dissertation. The defense occurred in Bush Auditorium, where James D. Watson first publicly presented the structure of DNA 50 years earlier. Present as the external examiner was Nobel laureate Phillip Sharp, an alum of Cold Spring Harbor Laboratory. It was a truly momentous occasion. The idea of a graduate school at Cold Spring Harbor Laboratory was raised by Jim Watson in the fall of 1995. Just seven and a half years later, the Laboratory awarded its first Ph.D. degree. It now truly is a degree-granting institution.

Amy Caudy

And Amy Caudy set a benchmark for many to follow. As a Howard Hughes Medical Institute Predoctoral Trainee and in the process of her doctoral research in Gregory Hannon's laboratory, she participated in seven published studies and was the primary author on publications in *Genes & Development* and *Nature*, all pertaining to her research on mechanisms of RNA interference or RNAi. But that was not enough for Amy. She was also a coauthor on publications arising from the research she performed in each of two six-week research rotations during her first year of study. It was perhaps not surprising—but certainly gratifying nonetheless—that, as described further below, Amy was awarded the Harold Weintraub award by the Fred Hutchison Cancer Research Center.

Amy is now at Washington University in St. Louis for her postdoctoral studies in immunology with John Atkinson. She is also coauthor of a new revision of the book *Recombinant DNA* together with James Watson, Richard Myers, and Jan Witkowski. Then again, all of this success is perhaps not that surprising—when in college, Amy was selected as one of the top ten women undergraduates of 1998 by *Glamour* Magazine!

Amy Caudy was not the only Watson School student to graduate this year. This fall, Ira Hall defended his thesis for his studies with Shiv Grewal after only just over three years in the School. We were most pleased that our colleague Rolf Sternglanz from Stony Brook University participated as the external examiner. Cold Spring Harbor Laboratory faculty members have participated in Stony Brook University thesis defenses for more than 25 years, so it was truly a pleasure to have the roles reversed! Like Amy, Ira Hall has been extremely successful as a student. During his doctoral studies, Ira coauthored two publications that were cited as the foundation of the 2002 "Breakthrough of the Year" by *Science* magazine. These two publications have also just this fall been awarded the Newcomb Cleveland Award for being the most important publications in *Science* magazine in 2002. Impressive achievements indeed!



Ira Hall

A very pleasing fallout to the Watson School's involvement in thesis defenses has been our becoming more engaged in promoting the thesis defenses of students from other institutions, particularly Stony Brook University. Thus, whereas in the past, thesis defenses were often poorly publicized and attended, the Watson School now ensures that they are announced labwide and are often held in Bush Auditorium where attendance has been outstanding. This development is just one example of how Cold Spring Harbor Laboratory is transforming itself into an academic institution.

The first Watson School graduation is scheduled for April 25, 2004—exactly 51 years after the publication of the structure of DNA by Watson and Crick! Amy and Ira are likely to be joined by other Watson School graduates at what will be a most festive occasion.

THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 1999

Amy A. Caudy, May 13, 2003

Mechanism and Applications of RNA Interference

Thesis Examining Committee

Chair:	**Adrian R. Krainer**
Research Mentor:	**Gregory Hannon**
Academic Mentor:	**Hollis Cline**
Committee Member:	**Robert Martienssen**
Committee Member:	**Bruce Stillman**
External Examiner:	**Phillip A. Sharp,** Massachusetts Institute of Technology

ENTERING CLASS OF 2000

Ira M. Hall, November 20, 2003

A Role for RNA Interference in Heterochromatic Silencing and Chromosome Dynamics

Thesis Examining Committee

Chair:	**Rui-Ming Xu**
Research Mentor:	**Shiv Grewal**
Academic Mentor:	**Alexander A.F. Gann**
Committee Member:	**Scott Lowe**
Committee Member:	**Michael Wigler**
External Examiner:	**Rolf Sternglanz,** Stony Brook University

Student Awards

When the Watson School established as a founding principle the goal of offering a four-year Ph.D. degree in the biological sciences, one concern raised by all involved in establishing the School was whether it would be possible for students to perform substantive research within such a timeframe. Now, just over four years since the first students entered the School, the early results suggest that it is imminently possible and how! The first two Watson School students to defend their theses, Amy Caudy and Ira Hall, have received international recognition for their doctoral studies.

Amy celebrating a four-year Ph.D.

Amy Caudy Receives the Harold M. Weintraub Graduate Student Award

This spring, Amy Caudy was one of 16 graduate students from North America and Europe to be selected to receive the 2003 Harold M. Weintraub Graduate Student Award, which is sponsored by the Fred Hutchinson Cancer Research Center. The award, established in 2000, honors the late Harold M. Weintraub, Ph.D., a founding member of Fred Hutchinson's Basic Sciences Division, who in 1995 died from brain cancer at age 49. Weintraub was an international leader in the field of molecular biology. Among his many contributions, he identified genes responsible for instructing cells to differentiate, or develop, into specific tissues such as muscle and bone. Nominations for the award were solicited internationally, and the winners were selected on the basis of the quality, originality, and significance of their work. Amy received her award for her seminal studies on the mechanisms of RNAi.

Ira Hall's Studies Recognized by the American Association for the Advancement of Science

During his doctoral studies, Ira Hall participated in two collaborative studies that were recognized in 2002 by the journal Science as the "Breakthrough of the Year." This year, the American Association for the Advancement of Science (AAAS) recognized these two studies with the award of the 2003 Newcomb Cleveland Prize. The AAAS Newcomb Cleveland Prize acknowledges an outstanding paper published in the journal *Science*. It is the oldest and largest of the AAAS awards and was established in 1923. The AAAS noted that the two papers recognized in the 2003 Newcomb Cleveland Award established a new landmark in epigenetic control. They added a giant step to our knowledge by showing how RNA molecules directly control gene expression by modifying chromatin structure and func-

tion. The AAAS noted that these elegant experiments have laid the basis for new understanding of a wide range of "textbook" processes that have arisen from classical genetic studies, including imprinting and dosage compensation through X-inactivation.

One of the reasons for the success of researchers at Cold Spring Harbor Laboratory is the high degree of interaction and collaboration among its scientists. Ira's studies are no exception. They involved collaborations with other members of Shiv Grewal's laboratory and in one of the two cases with members of Robert Martienssen's laboratory.

Catherine Cormier Receives a National Science Foundation Graduate Research Fellowship

In addition to the recognition of graduating Watson School students' research, Catherine Cormier (entering class of 2001) became the first Watson School student to receive the National Science Foundation (NSF) Graduate Research Fellowship. This year, the Howard Hughes Medical Institute regrettably terminated its prestigious predoctoral fellowship program, a program that awarded four of its fellowships to Watson School students: Amy Caudy, Emiliano Rial Verde, Dougal (Gowan) Tervo, and Elizabeth Thomas. Now that this program has been terminated, it is our hope that more students will be awarded the NSF fellowship like Cathy.

Catherine Cormier

Recruiting a 2003 Entering Class

Each year, the Watson School puts itself forward in the hopes that the best and the brightest will select it for their doctoral studies in the biological sciences. In 2003, these hopes were satisfied. Of 13 offers of admission made by the Watson School to applicants, 8 were accepted—a greater than 60% acceptance rate. Students turned down offers from University of California, Berkeley; Massachusetts Institute of Technology; and Stanford University, among others, to join the Watson School. The five students who went elsewhere decided to go to prestigious institutions: Cambridge University (U.K.), Harvard University, Massachusetts Institute of Technology, The Rockefeller University, and University of California at San Francisco. With one student deferral from 2002, there were nine entering students this year (see photo and box), more than our original goal of seven to eight students. The increased size of the 2003 entering class required more effort by the teaching faculty, but this change was most welcome!

The recruitment of a new class is the result of the efforts of the Admissions Committee, Watson School administration, faculty, and students. The School is most appreciative of Nouria Hernandez' efforts these past three years as chair of the Admissions Committee. Her dedication to the recruitment effort is an important reason for this year's success. Her position as chair of the Admissions Committee has now been filled by Marja Timmermans. The School looks forward to continued recruitment successes under her direction, especially with the help of Ms. Dawn Meehan, the School's new recruitment specialist (see below).

The Fifth Entering Class

It is hard for me to believe that it is only four years ago that the Watson School first opened its doors to students. I still remember vividly Lilian Gann and I working until late in the evening of September 6, 1999 to get things ready for that first entering class. This year, on August 25, the Watson School opened its doors for the fifth time to welcome a new class of students. Nine students–Hiroki Asari, Rebecca Bish, François Bolduc, Monica Dus, Angélique Girard, Christopher Harvey, Jeong-Gu Kang,

Izabela Sujka, and Wei Wei—all began together their adventure in learning how to do science and become scholars in the biological sciences. As is typical for the Watson School, the entering students hailed from around the world (see box). With nine students, this class matches in size our previously largest class, the entering class of 2000. We hope that this larger size portends well for the Watson School beginning to have on the order of 10–12 students per year.

Student Amenities

To attract such outstanding students, some important changes were made this year in student amenities. First and foremost, the Watson School student annual stipend increased from $21,500 to

DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
ENTERING CLASS OF 1999			
Amy Anne Caudy *George A. and Marjorie H. Anderson Fellow* *Howard Hughes Medical Institute Predoctoral Fellow* **Thesis defense: May 2003**	Hollis Cline	Gregory Hannon	The biological function of RNA interference
Michelle Lynn Cilia *William R. Miller Fellow*	Nouria Hernandez	David Jackson	Mechanisms of intercellular trafficking via plasmodesmata
Ahmet M. Denli *David H. Koch Fellow*	Adrian R. Krainer	Gregory Hannon	Biochemical analysis of RNA-induced gene silencing
Emiliano M. Rial Verde *David and Fanny Luke Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Jan A. Witkowski	Hollis Cline	Arc role in synaptic function
Elizabeth Ellen Thomas *Farish-Gerry Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	William Tansey	Michael Wigler	A de novo approach to identifying repetitive elements in genomic sequences
Niraj H. Tolia *Leslie C. Quick, Jr. Fellow*	David Helfman	Leemor Joshua-Tor	Structural analysis of a malarial surface antigen
ENTERING CLASS OF 2000			
Santanu Chakraborty *George A. and Marjorie H. Anderson Fellow*	Michael Wigler	Carlos D. Brody	Mechanisms of robust short-term memory in biological networks
Elena S. Ezhkova *Engelhorn Scholar*	Jan A. Witkowski	William Tansey	Role of the proteasome in transcription
Rebecca C. Ewald *Engelhorn Scholar*	Bruce Stillman	Hollis Cline	NMDA receptor trafficking and its impact on neuronal functionality and morphology
Ira Hall *Beckman Graduate Student* **Thesis defense: May 2003**	Alexander A.F. Gann	Shiv Grewal	Initiation, assembly, transfer, and genome-wide distribution of heterochromatin in *Schizosaccharomyces pombe*
Zachary Bela Lippman *Beckman Graduate Student*	William Tansey	Robert Martienssen	Comprehensive analysis of chromatin status on *Arabidopsis* chromosome 4
Marco Mangone *Charles A. Dana Foundation Fellow*	Linda Van Aelst	Winship Herr	The role of HCF-1 in cell proliferation
Masafumi Muratani *George A. and Marjone H. Anderson Fellow*	Nouria Hernandez	William Tansey	Gene regulation by ubiquitin-mediated proteolysis
Patrick J. Paddison *Beckman Graduate Student*	Adrian R. Krainer	Gregory Hannon	An RNAi-based screen in mouse embryo fibroblasts for transformation-lethal gene targets

$25,000. This stipend level is more in line with those of the schools with which we compete for students. In addition to stipends, Cold Spring Harbor Laboratory has made serious efforts at improving housing accomodations for all students and postdocs. A new Housing Office has been established in the Facilities Department. Additionally, owing to the support of the Board of Trustees, excellent housing is being renovated and constructed de novo at the Uplands Farm site, providing living space for 32 students and postdocs. Already four Watson School and two Stony Brook University students are sharing the first renovated residence at the site. As an indication of the importance of housing to the Watson School, I became chair of a housing committee to establish a defined housing program for the Laboratory. I have enjoyed working closely with Art Brings, the Laboratory's Chief Facilities Officer, on this very important issue.

DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
ENTERING CLASS OF 2001			
Catherine Y. Cormier *Beckman Graduate Student*	David J. Stewart	Yuri Lazebnik	Caspase activation in unidentified apoptotic pathways
Claudia E. Feierstein *George A. and Marjorie H. Anderson Fellow*	Linda Van Aelst	Zachary Mainen	Odor coding and neural correlates of behavioral choice in the olfactory cortex
Tomáš Hromádka *Engelhorn Scholar*	William Tansey	Anthony Zador	Stimulus optimization in the auditory cortex
Charles D. Kopec *Goldberg-Lindsay Fellow*	Anthony Zador	Roberto Malinow	Mapping the trafficking of AMPA receptors in dendritic compartments
Ji-Joon Song *Bristol-Myers Squibb Fellow*	Scott Lowe	Leemor Joshua-Tor	Structural studies of RNAi
Dougal G.R. (Gowan) Tervo *George A. and Marjorie H. Anderson Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Carlos D. Brody	Karel Svoboda	An inducible and reversible lesion of the corticothalmic projection
ENTERING CLASS OF 2002			
Allison L. Blum *Barbara McClintock Fellow*	Leemor Joshua-Tor	Josh Dubnau	Genetic, behavioral, and anatomical characterization of Radish-dependent memory
Darren Burgess *Engelhorn Scholar*	Nicholas Tonks	Scott Lowe	Mammalian RNAi genetic screens: Discovery and characterization of genes mediating the response to cancer therapy
Beth L. Chen *George A. and Marjorie H. Anderson Fellow* *Beckman Graduate Student*	Senthil K. Muthuswamy	Dmitri Chklovskii	Neuronal network of *C. elegans:* From anatomy to behavior
Shu-Ling Chiu *Elisabeth Sloan Livingston Fellow*	Alea A. Mills	Hollis Cline	To be determined
Jonathan Kui *Alfred Hershey Fellow*	David Jackson	Tim Tully	Identification and characterization of candidate memory genes in Arleekin, a *Drosophila* memory mutant
Elizabeth Murchison *Elisabeth Sloan Livingston Fellow*	John Inglis	Gregory Hannon	The role of Dicer in mammalian development

ENTERING CLASS OF 2003

Hiroki Asari, The University of Tokyo, Japan
Farrish-Gerry Fellow

Rebecca A. Bish, Massachusetts Institute of Technology
David H. Koch Fellow

François Bolduc, M.D., Université de Sherbrooke, Canada; Université de Montreal, Canada; McGill University, Canada
William R. Miller Fellow

Monica Dus, University of Redlands, California
Engelhorn Scholar

Angélique Girard, École Polytechnique, France; Corps des Mines, France
Florence Gould Fellow

Christopher D. Harvey, Vanderbilt University, Tennessee
David and Fanny Luke Fellow

Jeong-Gu Kang, Seoul National University, Korea
Watson School Fellow

Izabela A. Sujka, Stony Brook University, New York
Beckman Graduate Student

Wei Wei, University of Melbourne, Australia
Leslie C. Quick, Jr. Fellow

Standing (left to right): Rebecca Bish, François Bulduc, Monica Dus, Izzabela Sujka, Hiroki Arari.
Seated (left to right): Christopher Harvey, Angélique Girard, Wei Wei, Jeong-Gu Kang.

WATSON SCHOOL OF BIOLOGICAL SCIENCES 2003
UNDERREPRESENTED MINORITY RECRUITMENT EVENTS

Greater Baltimore Consortium Graduate School Fair
Loyola College, Baltimore, Maryland
September 22, 2003

Society for Advancement of Chicanos and Native Americans in Science (SACNAS)
Albuquerque, New Mexico
October 2–5, 2003

Annual Biomedical Research Conference for Minority Students (ABRCMS)
San Diego, California
October 14–18, 2003

University of Maryland, Baltimore County
Meyerhoff Program, Baltimore, Maryland
October 29, 2003

State University of New York Manhattan Career Day Fair
SUNY Manhattan, New York City
November 5, 2003

Ronald E. McNair Scholars Conference
Milwaukee, Wisconsin
November 7–9, 2003

National Association of African American Honors Program (NAAAHP)
Orlando, Florida
November 13–15, 2003

Recruitment of Students from Underrepresented Minorities

The Watson School is dedicated to the recruitment, support, and success of individuals from underrepresented minority groups in the biological sciences. The School is taking a very active role in ensuring success in this endeavor as evidenced by the large number of underrepresented minority recruitment events attended by the Watson School this fall (see box). In the most significant event, October saw the Watson School staff go in full force (only Postdoctoral Program Officer Alyson Kass-Eisler stayed behind to hold down the fort!) to the Annual Biomedical Research Conference for Minority Students (ABRCMS) in San Diego, where it manned a booth. Dawn Meehan put together a slide show of Cold Spring Harbor Laboratory and the Watson School, which—along with Jim Watson's picture on display!—attracted much attention. We were very pleased to learn by year's end that these efforts resulted in a fourfold increase in the number of underrepresented minority applicants to the Watson School.

In addition to the conferences, I visited the Meyerhoff Program, an elite undergraduate program involving many first-rate minority students at the University of Maryland, Baltimore County (UMBC). I had on this occasion the opportunity to meet with UMBC President Freeman Hrabowski III, who was responsible for establishing the Meyerhoff Program. One of our topics of discussion was how to mentor students to success in their doctoral studies. Dr. Hrabowski was very interested in the Watson School's extensive mentoring programs.

As has so often been the case in the past, the Undergraduate Research Program is aiding the School to recruit underrepresented minority students. Last summer, a participant from the Meyerhoff Program, Keisha John, worked with Hollis Cline. Keisha generously helped us recruit students to the Undergraduate Research Program at our booth at the ABRCMS conference in San Diego. She additionally hosted me during my visit to the UMBC campus. We were very happy that Keisha was the first student to have a completed application to the Watson School this fall. And we are even more excited to learn, as of this writing, that she will be joining us in the Fall 2004 for her doctoral studies.

William Randolph Hearst Scholars Award

This year, with the support of the William Randolph Hearst Foundation, the Watson School established the William Randolph Hearst Scholars Award for exceptional Watson School doctoral candidates from underserved or underrepresented groups. The philanthropist William Randolph Hearst established the William Randolph Hearst Foundation in 1945. William Randolph Hearst was born on April 29, 1863, in San Francisco, California. In 1887, at 23 he became "Proprietor" of the *San Francisco Examiner*, which his father, George Hearst, accepted as payment for a gambling debt! He went on to be a newspaper magnate.

The William Randolph Hearst Foundation provides education opportunities in science at the undergraduate and graduate levels. In particular, it assists institutions in supporting students from underserved and underrepresented populations and, with this in mind, has provided the Watson School with an endowment to support such students.

The William Randolph Hearst Scholars Award provides the recipient with $2500 for each of four years in the form of an educational supplement. The funds are to further the recipient's graduate studies, and their use is specifically designed to be flexible. The School hopes that this award will help diversify its student population, an important national imperative.

Visit to Warsaw and France

The United States was not the only country the School visited this year. In May, I took advantage of the thesis defense of Joanna Wysocka, a student from the University of Warsaw who performed her doctoral research in my laboratory, to visit Poland. I was given the opportunity to meet with faculty and to describe the Watson School to undergraduates. I also made similar presentations in a tour of French schools—the Claude Bernard University of Lyon, the École Normale Supérieure in Lyon, and the École Polytechnique outside Paris. The visit to the École Polytechnique followed on my visit in July 2002 to participate in Angélique Girard's—the current Watson School Florence Gould Fellow—description of her "Stage d'Option" (a research internship) studies in my laboratory that spring. This time, I had an opportunity to meet directly with the third-year students. It must have gone well. We will have not just one but two students from the École Polytechnique for the Stage d'Option this upcoming spring.

Curriculum

By being a small school and at a flexible institution like Cold Spring Harbor Laboratory, the Watson School can offer a well-integrated curriculum, but it needs to be so organized. To aid in the organization of the curriculum, last spring saw the establishment of the Curriculum Development and Integration Committee (CDIC) with the charge to integrate the course instruction and monitor and balance student workload, particularly during the fall course term. I am pleased to report that, in its first year, this committee, consisting of William Tansey (Chair), Lilian Gann, David Spector, and Nicholas Tonks, had a significant impact on equilibrating the student workload this fall. In past years, there was often an occasion during the biweekly Dean's Teas of the fall term that the students would complain to me about work overload. With the improved equilibration engineered by the CDIC, I am happy to report that there were no tumultuous Dean's Teas this fall!

The CDIC additionally solved a long-standing problem with the Watson School curriculum by recruiting Tim Tully to develop and lead a new course on Genetics co-taught by Josh Dubnau and Lincoln Stein. The course was very highly rated by the students. Its success addressed a recurring issue raised by the School's External Advisory Committee on how to teach our students genetics effectively. It is very pleasing to see the success brought by the involvement of new faculty in the School's curriculum.

A Complete Set of Topics in Biology Courses

This spring saw the fourth Topics in Biology course presented by the Watson School—a one-week-long course organized by Jan Witkowski and Lilian Gann and taught at the Banbury Center. H. Kern Reeve from Cornell University taught a course on animal behavior. With this course, the Watson School completed a set of four Topics in Biology courses to be taken by students in each of their four years in the School: Immunology, Evolution, Microbial Pathogenesis, and Animal Behavior (see box). This year, Nipam Patel, now at the University of California, Berkeley, also returned to teach his course on evolution. Both the new Animal Behavior course and returning Evolution course were well received by both students and instructors alike. These results make the Topics in Biology courses among the most successful and very special parts of the Watson School curriculum.

FOUR *TOPICS IN BIOLOGY* COURSES

ANIMAL BEHAVIOR
H. Kern Reeve, Cornell University

EVOLUTION
Nipam Patel, University of California, Berkeley

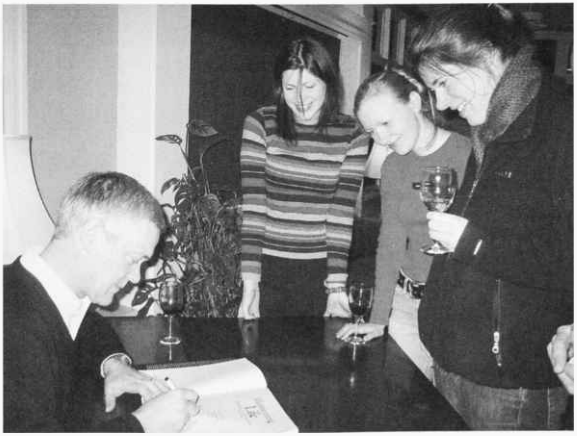
IMMUNOLOGY
Hidde Ploegh, Harvard Medical School

MICROBIAL PATHOGENESIS
Stanley Maloy, San Diego State University
Ronald Taylor, Dartmouth College

Graduate Student Seminar Series

One of the most important but often overlooked elements of the graduate student experience at Cold Spring Harbor Laboratory is the weekly Graduate Student Seminar Series. The Laboratory is special in the way it has a diverse multi-institutional graduate student community, especially mixing Stony Brook University and Watson School students. The graduate student seminars, which are held each Tuesday evening from September to May over dinner (kindly arranged by Yvette Seger), bring all graduate students studying at the Laboratory to hear seminars from their colleagues.

Students present their research once a year, with two students presenting each week. In attendance are the graduate students and two members of a core set of four faculty mentors whose attendance rotates each week. The seminar series serves three important roles. First, it gives students an opportunity to hone their oral presentation skills. Second, it provides the students with an opportunity to defend their research in the absence of their research mentors, such that they experience standing on their own two feet as they will have to throughout their careers. Third, the students in the audience have an opportunity to ask questions within their own peer group, thus learning the important roles of audience participation for the advancement of research. At the end of the evening, the two faculty mentors provide each presenting student a critique of the seminar and sometimes provide feedback to the student's research mentor.



Illuminating Life book signing
Left to right: Jan Witkowski, Monica Dus, Izabela Sujka, and Elizabeth Murchison

This seminar program has been spearheaded and organized for many years by David Helfman with the help of Madeline Wisnewski. David has been steadfast in insisting that students have a scholarly approach to their education and take an interest in a broad range of subjects as well as in their colleagues' welfare. David stepped down from this important role this year and handed over the organizational responsibilities to Mark Beavers and me in the Watson School, a change that emphasizes the importance the School places on this seminar series. Terri Grodzicker, Alea Mills, and Arne Stenlund continue as faculty mentors, and, to fulfill David Helfman's role, they are joined by our new faculty member Josh Dubnau. Josh brings a broad range of expertise in genetics, molecular biology, and neurobiology.

Academic Mentoring

The Watson School takes great pride in the level of mentoring that it offers to its students. One of the very special aspects in this regard is the Watson School academic mentoring program, led by William Tansey. In this program, entering students select by mutual agreement a member of the research or nonresearch faculty to serve as an academic mentor—a guardian angel to look over and encourage the student through the sometimes trying process of a doctoral education. This program continues to receive much support from the faculty who volunteer to be academic mentors, and it has rightfully become a vital ingredient in the Watson School's success. This year's new academic mentors for the entering class of 2003 are:

Hiroki Asari	Z. Josh Huang
Rebecca Bish	Linda Van Aelst
François Bolduc	Hollis Cline
Monica Dus	John Inglis
Angélique Girard	Nouria Hernandez
Christopher Harvey	Adrian R. Krainer
Jeong-Gu Kang	Scott Lowe
Izabela Sujka	Marja Timmermans
Wei Wei	Jan A. Witkowski

New Faculty

Four new research faculty joined the Laboratory this year. Josh Dubnau, who came to Cold Spring Harbor Laboratory as a postdoctoral fellow with Tim Tully, became an assistant professor at the beginning of the year and immediately became involved in the Watson School. He was an instructor in the highly acclaimed Fall Term Genetics course led by Tim Tully and became a faculty mentor in the graduate student seminar series. He is also Allison Blum's research mentor. Josh studies learning and memory in the fruit fly *Drosophila melanogaster*.

Josh was joined during the year by two other neurobiologists: Alexei Koulakov as assistant professor and Partha Mitra as professor. Both Alexei and Partha use computational methods to address how the brain works. At the end of the year, the most recent Cold Spring Harbor Laboratory recruit, Wolfgang Lukowitz, joined the faculty as assistant professor in the plant genetics group. Wolfgang studies cell division polarity in the early plant embryo.

With all these comings, regrettably, there was also someone going. Shiv Grewal, who had great success in his studies of epigenetic control of gene expression in the fission yeast *Schizosaccharomyces pombe* at the Laboratory, became Principal Investigator at the National Cancer Institute. I, in particular, felt the loss caused by his departure as Shiv and I had co-instructed the Chromosome Structure and Function week of the Scientific Reasoning and Logic course for the past two years. Shiv was sorely missed this fall when I had to teach the same topic. Fortunately, I was rescued by Shiv's student Ira Hall, who gave a lovely lecture on *S. pombe* epigenetic inheritance.

Graduate Student and Postdoctoral Fellow Departures

With each year come not only new arrivals, but also new departures. This year saw the following graduate students and postodoctoral fellows depart from the Laboratory.

Graduate Students

Anitra Auster
Brian Chen
Brian Hill
Asra Malikzay
Thomas Tubon
Emily Bernstein
Amy Caudy
Ajit Janardhan
John Mignone
Joanna Wysocka
Alyssa Carlberg
Carmine Chiariello
Edward Kim
Zaher Nahle

Postdoctoral Fellows

Katherine Braun
Kim L. Farina Graham
Naoya Hata
Guy A. Karger
Patrice Lassus
Kenichi Noma
Xiaokang Pan
Tomasz Swigut
Erik W. Vollbrecht
Canzhu Yang
Oliver Cuvier
Toshiyuki Fukada
Songtao Jia
Jae-Yean Kim
Tzu-Ching Meng
Thomas Oertner
Geraldine Pawlak
Joshua T. Trachtenberg
Thomas Volpe
Zhiguo Zhang
Veronica Egger
Ivo Grosse
Eric Julien
Rajeev Kumar
Esther Nimchinsky
Helena L. Palka-Hamblin
Kenneth J. Seidenman
Anne Vaahtokari
Qun Wang

Partners for the Future Program

The Watson School oversees educational programs that extend from the Nature Study Program for youngsters to the postdoctoral training program. The oldest of the programs in the Watson School, the Nature Study Program, runs on its own without intervention from the Watson School. In contrast, the Partners for the Future Program, a collaboration between the Watson School and the Public Affairs Department, has benefited in recent years from the efforts of Yuri Lazebnik, who has taken a hands-on approach to increase the educational value of the program. The Partners for the Future Program provides high school students an opportunity to experience laboratory research. High school seniors work side by side with postdoctoral fellows or professors from October to March. Changes instigated by Yuri to increase camaraderie among the high school students include get-togethers at which the students present their research through oral presentations. These get-togethers offer the students an opportunity (1) to learn how to explain their research efforts, (2) to expand their awareness of different research areas through their colleagues' presentations, and (3) to get to know one another. Yuri's contributions to the Partners for the Future Program are only one example of how some of the Laboratory's faculty contribute to the academic environment of the Laboratory with the only reward being the knowledge that they may influence the early careers of future scientists.

Undergraduate Research Program

The summer Undergraduate Research Program continues to be a cornerstone of the Watson School. This year, nearly 500 students applied to the program (476 to be exact!) from across the country and around the world. The participants—called URPs, participate in the ten-week program extending from the second week of June to the middle of August. They possess a strong esprit de corps that brings the closest thing to an undergraduate feel to the Laboratory.

This program, together with its offshoot, the Undergraduate Scholars Exchange Program, which provides undergraduate research opportunities during the nonsummer months, also continues to be a

WSBS student Elena Ezhkova (*left*) and URPS Jonathan Schneiderman, Keisha John, Catherine Del Vecchio, and Galen Collins (*left to right*) modeling *DNA Stuff* merchandise.

rich source of students for the graduate school. Thus, in this year's entering class, Hiroki Asari was an URP, and Angélique Girard, Wei Wei, and François Bolduc were exchange scholars. Once students get a sense of the wonderful interactive intellectual community at the Laboratory, it is much easier to convince them to return for their doctoral studies. Given the importance of the Undergraduate Research Program to the graduate school, this year I met all of the 25 URPs, five at a time, for lunch in Blackford Hall. The discussions were wonderful. These lunches were among my favorite part of being dean last year. Fortunately, by year's end, seven former URPs had applied to the School for admission in the fall 2004.

The program this summer was again expertly directed by Leemor Joshua-Tor, who has had a major impact on the excellence of the program and developed new elements for it. Indeed, it was in discussions with Leemor about how I might get to know the URPs better that the idea of the lunches came up. Her dedication to the program has had a major impact on the Watson School and the Laboratory in general. Unfortunately, as this year's summer season came to a close, Leemor decided that she needed to focus more on her research and therefore has taken a leave of absence from being director. Fortunately, Senthil Muthuswamy has agreed to take over for this year. I am confident that, with his energy and outstanding abilities, the program will continue to thrive.

Postdoctoral Program

As announced last year, a new postdoctoral program was initiated at the beginning of this year directed by Nicholas Tonks together with Associate Dean Lilian Gann and our new Postdoctoral Program Officer Alyson Kass-Eisler, the mainstay of the postdoctoral program operation. The postdoctoral program arose from the efforts of a postdoctoral working group, which recommended that the Laboratory take a more hands-on approach to the education of postdoctoral fellows. Importantly, with the help of the Postdoctoral Program Office, the resident postdoctoral fellows established a Cold Spring Harbor Laboratory Postdoctoral Association. This "Postdoc" Association has organized subcommittees focused on such issues as career development, housing, postdoc Web site, immigration, and general welfare. An important benefit of the Postdoc Association is that it empowers Laboratory postdoctoral fellows with the ability to influence their own collective experience at the Laboratory.

In its first year, the Postdoctoral Program Office has rapidly established the role of aiding new post-

doctoral fellows to identify fellowship opportunities to support their research. The preparation of applications for postdoctoral fellowships is an integral part of the postdoctoral experience. Besides potentially providing financial support, the postdoctoral fellowship application is an outstanding mechanism for postdoctoral fellows and their mentors to become actively engaged in defining a research program that will lead to a successful postdoctoral research experience. There are a myriad of postdoctoral fellowship opportunities that are appropriate for different fellows. By developing—and now maintaining—a coherent and well-organized database, Alyson has greatly enhanced the ability and ease with which new postdoctoral fellows can identify potential fellowship opportunities. Such efforts will help the Laboratory finance its research program.

For me, one of the most exciting aspects of the establishment of the Watson School has been how its doctoral program can take advantage of all the diverse elements of Cold Spring Harbor Laboratory. This aspect has now extended to the postdoctoral program. One of the recent efforts of the Cold Spring Harbor Laboratory Press has been in the realm of publishing books that deal with the practical aspects of doing science. Thus, in collaboration with the author Kathy Barker, the Laboratory Press has produced two books—*At the Bench* and *At the Helm*—that deal with how to do research at the bench and how to lead a research laboratory. The Postdoctoral Program has taken advantage of this effort by providing, with financial support from the Laboratory President Bruce Stillman, each postdoctoral fellow at the Laboratory with a copy of these two books. Additionally, Alyson Kass Eisler was able to get Kathy Barker herself to present a seminar entitled "Lessons from labheads: Making your lab work for you," which covered aspects of directing a research laboratory. Such efforts greatly aid the future success of the Laboratory's postdoctoral fellows.

Perhaps most important to postdoctoral fellows is developing the wherewithal to obtain a job after the postdoctoral experience. With this in mind, the Postdoctoral Program Office sponsored a workshop on the mechanisms of obtaining a research grant. Thus, Adrian Krainer and Grants Department Director Susan Schultz described how a research grant, especially one submitted to the National Institutes of Health, is reviewed and funded. By emphasizing the mechanics of how grants are reviewed, it was also possible to describe how to best prepare the written document.

Alyson also had the idea to arrange a very interesting series of get-togethers in the spring for postdocs to share their experiences in searching for jobs. There is no better to way to learn but to hear from those who have firsthand experience. Obtaining a job as a research leader, particularly an academic position, requires a diverse set of talents from presenting an interesting research seminar, to engaging interviewing faculty during one-on-one discussions, to negotiating an offer. By sharing experiences, postdoctoral fellows who had been on the "job circuit" could pass along their acquired expertise. What is so special to me about this aspect of the newly developed postdoctoral program is that by simply getting people together to share their acquired knowledge, we are able to enhance the postdoctoral experience at the Laboratory.

This year, Alyson helped me inaugurate a new custom in the Watson School. Now, about three times a year, I invite new postdoctoral arrivals to join me for tea to discuss the postdoctoral experience at the Laboratory. At these teas, I get to describe my own experience as a postdoctoral fellow at the Laboratory—some 20 years ago!—and how to take advantage of all the very special resources of the Laboratory, from the Meetings and Courses to the Blackford Bar! These teas also provide an opportunity for the new postdocs to identify their colleagues who are also recent arrivals, colleagues with whom they can share their experiences as they progress as postdoctoral fellows at the Laboratory. And I likewise get to know members of the largest segment of the Laboratory's research community. In this vein, I was very pleased that Bruce Stillman could continue having his annual meeting with all of the postdoctoral fellows at the Laboratory in the fall. These annual meetings provide postdoctoral fellows with an opportunity to influence the direction of the Laboratory. Indeed, the Postdoctoral Program Office has its origins in early postdoctoral meetings between Bruce Stillman and postdoctoral fellows.

These are good beginnings, but the Laboratory needs to develop a more coherent postdoctoral training program. With this concern in mind, a second postdoctoral working group was established this year to recommend the structure of a comprehensive postdoctoral training program. I was very pleased to recruit a strong group of faculty, postdoctoral fellows, and Watson School staff for the

NEW STUDENTS FROM SHARED STONY BROOK UNIVERSITY GRADUATE PROGRAMS

Student	CSHL Research Mentor	Program
Kasandra Burgos	Hollis Cline	Genetics
Pei-Chun Lin	Rui-Ming Xu	Genetics
Christopher Maher	Lincoln Stein	Biomedical Engineering
Cristian Papazoglu-Statescu	Winship Herr	Genetics
Avi Z. Rosenberg	Senthil Muthuswamy	Genetics
Marlies Rossmann	Bruce Stillman	Genetics
Dustin Schones	Michael Zhang	Physics
Despina Siolas	Gregory Hannon	Genetics
Aleksander Sobczyk	Karel Svoboda	Physics
Zankhana Thakkar	Jacek Skowronski	Molecular Genetics and Microbiology
Quan Wen	Dmitri Chklovskii	Physics

committee. In addition to me as chair, they include Lilian Gann and Alyson Kass-Eisler from the Watson School, postdoctoral fellows Kurt Haas, Michael Hemann, Sarah Newey, Vatsala Thirumalai, and Michael Weher, and faculty members Leemor Joshua-Tor, Adrian Krainer, Senthil Muthuswamy, and Nicholas Tonks. The committee will present a series of recommendations to the Laboratory president by summer 2004.

Interinstitutional Academic Interactions

Cold Spring Harbor Laboratory relies heavily on good interactions with neighboring academic institutions, particularly Stony Brook University. To enhance these interactions, I hosted individual lunches with Lawrence Martin, Dean of the Graduate School, and Peter Gergen, Todd Miller, and Michael Frohman, who lead the Genetics, Molecular and Cellular Biology, and M.D./Ph.D. programs, respectively. In addition to helpful discussions over lunch, these meetings also provide the program leaders an opportunity to meet with their respective students.

It is important to always keep in mind that most of the graduate students studying at the Laboratory are not Watson School students. Indeed, the largest percentage of students are from Stony Brook University. It is therefore important to make sure that these students feel a part of the Laboratory community and to help them as much as possible with the difficulties of performing doctoral research away from their parent institutions. This year, Lilian Gann and I held the first of what will become an annual "How is it going?" meeting with the non-Watson School students to hear of any concerns. We had an open and frank discussion that Lilian and I found most helpful.

New York Academy of Sciences Alliance

In spring 2003, again at the generosity of the Laboratory's president Bruce Stillman, all graduate students and postdoctoral fellows at the Laboratory were enrolled in a new initiative of the New York Academy of Science, the Science Alliance. The Science Alliance for graduate students and postdoctoral fellows is a consortium of 14 universities, teaching hospitals, and independent research facilities in the New York City metro area that have formed a partnership with the New York Academy of Sciences. Through the Alliance, each institution sponsors Academy membership dues for all of its first-year graduate students and postdoctoral fellows. The aim of the Alliance is to provide career and professional development mentoring for graduate students and postdoctoral fellows in the sciences and engineering. Representatives from each of the participating institutions form a Steering Committee, which helps shape and develop Alliance events, programs, and Web content. Cold Spring Harbor

Laboratory's representatives are Zachary Lippman (graduate student) and Sarah Newey (postdoctoral fellow).

Executive Committee

A large measure of the Watson School's success can be traced to the sage advice and guidance of the School's Executive Committee. As the year came to a close, the Committee underwent some important changes. In 2002, I stepped down as the Laboratory's assistant director. Up until that time, I was able to wear two hats at the Executive Committee as both dean and assistant director. Fortunately, my role as assistant director was filled by Hollis Cline, who was already a member of the Executive Committee, so one hat only had to move around the table! This year, however, was the last of Holly's two three-year terms. Rather than lose the very important input of the Director of Research, the Executive Committee made the Director of Research an *ex officio* member of the Executive Committee, beginning in 2004. I am very pleased that the School will continue to receive Holly's valued input.

With the new year, the Executive Committee will lose a most valued member, David Helfman. David Helfman was one of those faculty who was very engaged in the Watson School's inception, joining in the original series of faculty teas at which the outlines of the Watson School were laid out. Throughout it all, among many contributions, David emphasized the importance of training scholars. He was a founding member of the Watson School's Executive Committee, and the School will sorely miss his involvement in the Executive Committee discussions. At year's end, the Executive Committee was pleased that W. Richard McCombie and Nicholas Tonks agreed to join the Committee as faculty members starting in 2004.

As happens each year, there was also turnover among the student representatives. Watson School representative Zachary Lippman was replaced by Catherine Cormier, and Stony Brook University representative Sabrina Nuñez was replaced by Marissa Moore. The School is indebted to their frank, honest, and thoughtful advice.

External Advisory Committee

Since its inception in 1998, the Watson School has relied heavily on advice from the External Advisory Committee (EAC). With the program reaching the time when it must begin having its students complete their thesis work in earnest to meet the four-year goal, I was most pleased that when the EAC, led by Keith Yamamoto, came in May for a site visit, they left feeling that the Watson School was in its best shape ever. Indeed, in pointing out that the Watson School is really showing itself, they mentioned that they were impressed with the remarkable number of Watson School concepts that were working as if "we knew all along that they would work!"

New Staff

The Watson School staff continued to evolve. This year, Janet Duffy, the founding Admissions and Academic Records Administrator, left the School to join the Facilities Department as Housing Manager. Although Janet left the School, in her important new role, she will still greatly aid the School by helping the graduate students and postdoctoral fellows with housing.

With Janet's departure, the Watson School recruited Dawn Meehan as the new Admissions and Curriculum Administrator. Dawn arrived with considerable experience in student recruitment. A graduate of Milton Academy and Wellesley College in Massachusetts, she spent time at both places in the admissions programs and with student recruitment. Thus, with Dawn's arrival, the School acquired firsthand experience from well-established academic institutions in the art of recruiting students. I have no doubt that Dawn will have a large impact on the success of the School by leading the effort to attract the very best students from across the nation and around the world to the School.

New Arrival

The Watson School had another "first" this year. One of its students, Zachary Lippman, was the first to have a child while enrolled in the School. His wife Shira gave birth to a lovely daughter, Nava Esther, on August 1, weighing in at 8 lb. 7 oz. Congratulations!

Nava Esther Lippman

The Watson School Continues to Benefit from Generous Benefactors

The Watson School has been fortunate to be the beneficiary of many generous benefactors who have provided support to the School's endowment for student, faculty, and administrative support. The School was particularly pleased this year to learn of Curt Engelhorn's continuing support of the Engelhorn Scholars Program with the appointment of Monica Dus, a 2003 Entering Class student who was born and raised in Italy. The Engelhorn Scholarships support European students in the Watson School and are essential to maintaining an exciting international student body in the Watson School.

I was most pleased to host Mr. Engelhorn and his daughter Carolin for a visit to Cold Spring Harbor Laboratory this fall, with the help of Vice President for Institutional Advancement Rod Miller. During the visit, Mr. and Ms. Engelhorn had an opportunity to meet four of the five Engelhorn Scholars, Monica, Darren Burgess, Rebecca Ewald, and Tomàš Hromàdka (unfortunately, the fifth Engelhorn Scholar, Elena Ezhkova, could not participate because she was stuck in Russia for four months waiting for renewal of her U.S. student visa!). We all greatly enjoyed the occasion.

WSBS Staff: (*Left to right*) M. Beavers, A. Kass-Eisler, D. Meehan, L. Gann, W. Herr

Dean Herr's 50th Birthday Celebration.

The Watson School has also benefited from the continued efforts of the Laboratory Trustees, particularly Robert Lindsay, who, having taken over from David Luke last year, is leading the second phase of the Watson School Campaign. To lay the groundwork for the second phase, Robert Lindsay hosted a series of dinners at the Links Club in Manhattan to introduce the Watson School to potential benefactors. I found the dinners most enjoyable as they offered benefactors an opportunity to meet students and faculty and to learn about what an exciting enterprise the Watson School is. All these development efforts were spearheaded and greatly aided by the efforts of Rod Miller and his staff, for which the School is indebted.

As we progress from one day to the next, dealing with each day one at a time, it is sometimes difficult to realize how much is actually accomplished in a year. Each year, preparing this report offers me an opportunity to reflect on the year's accomplishments. I very much hope that the readers of this report will agree that the Watson School continues to be an exciting adventure that is breaking new ground in doctoral education in the biological sciences. It is an adventure that is succeeding owing to the efforts of the faculty, students, administration, and the entire Cold Spring Harbor Laboratory institution and its friends. The upcoming inaugural graduation on April 25, 2004 will be a most special occasion to celebrate all these efforts.

Winship Herr
Dean

SPRING CURRICULUM

TOPICS IN BIOLOGY

ARRANGED BY **Lilian Gann**
Jan A. Witkowski

FUNDED IN PART BY **The Daniel E. Koshland, Jr. Visiting Lectureship; The David Pall Visiting Lectureship; The Fairchild Martindale Visiting Lectureship; The Lucy and Mark Ptashne Visiting Lectureship; The Michel David-Weill Visiting Lectureship**

Each year, one or a team of invited instructors offer seven-day courses at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the Cold Spring Harbor Laboratory Lecture Courses held each summer at the Banbury Conference Center. In spring 2003, there were two such courses: Evolution and Animal Behavior.

Evolution

Attended by the entering classes of 2001 and 2002

INSTRUCTOR **Nipam Patel,** University of Chicago

GUEST LECTURERS **Rob DeSalle,** American Museum of Natural History
Neil Shubin, University of Chicago
Michael Palopoli, Bowdoin College

TEACHING FELLOWS **Casey Bergman,** Lawrence Berkeley National Laboratory
Danielle Liubicich, University of Chicago
Marcus Davis, University of Chicago

Left to right: Gowan Tervo, Charles Kopec, Claudia Feierstein, Allison Blum, Casey Bergman, Shu-Ling Chiu, Jonathan Kui, Elizabeth Murchison, Catherine Cormier, Tomáš Hromádka, Danielle Liubicich, Marcus Davis, Darren Burgess, Nipam Patel, Beth Chen

The field of evolutionary biology touches upon all other areas of the biological sciences, since every form of life and every biological process represents an ongoing evolutionary "experiment." The aim of this course was both to discuss our understanding of the mechanisms of evolution and to explore how evolutionary data can be used to further our understanding of various biological problems.

The course ran from Sunday to Saturday, April 6–12, and was organized and largely taught by Nipam Patel. Three guests—Rob DeSalle, Neil Shubin, and Michael Palopoli—also lectured in the course, and three teaching fellows participated in all aspects of the course. As in previous years, the course was highly rated by all of the students

Animal Behavior

Attended by the entering classes of 1999 and 2000

INSTRUCTOR **H. Kern Reeve,** Cornell University

In this course, strong emphasis was placed on developing an understanding of the general principles governing the evolution of animal behavior, with particular emphasis on animal social behavior. The behavior of both vertebrate and invertebrate taxa was analyzed within this theoretical framework. Topics included group and individual-level selection, the units of behavioral selection, the evolution of sex, sexual selection (including intrasexual and intersexual competition), mating systems, kin selection and nepotism, game theory, reciprocity, communication, cooperative breeding and eusociality, reproductive skew within societies, and principles of cooperation and conflict between and within organisms.

The course ran from Sunday to Saturday, May 4–10 and was organized and solely taught by H. Kern Reeve. Once again, the course was highly rated by the students, many of whom had little prior awareness of the subject.

Left to right: H. Kern Reeve, Elizabeth Thomas, Zachary Lippman, Ahmet Denli, Elena Ezhkova, Santanu Chakraborty, Michelle Cilia, Ira Hall, Rebecca Ewald, Marco Mangone, Masafumi Muratani, Emiliano Rial Verde, Patrick Paddison, Niraj Tolia

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Scott Bronson**
Jenny Eisenman
Trisha Maskiell
Amanda McBrien

As science has an increasing role in society, there is also an increasing need for biologists to educate nonscientists of all ages about biology. The Watson School of Biological Sciences doctoral program offers its students unique teaching experiences through the Laboratory's Dolan DNA Learning Center, where students teach laboratory courses to high school and middle school students. From these teaching experiences, they learn how to communicate with nonbiologists and to inspire and educate creative young minds. The teaching module entailed pairs of students teaching one morning or afternoon a week for 12 weeks. In the initial weeks, the Dolan DNA Learning Center instructors taught the Watson School students the didactic process—it was not until the fifth week that the graduate students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty the opportunity to get to know each other and to explore possibilities for doctoral thesis research. At the end of each rotation, students make short presentations of their studies to the other students and their rotation advisors. These talks give students an opportunity to share their laboratory experiences and to learn how to give a scientific presentation. With this latter goal in mind, in addition to the research mentors, the instructors of the Scientific Exposition and Ethics core course attend the talks and give individual feedback to students on their presentations. This year, 14 faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS

Dmitri Chklovskii	**Scott Lowe**
Hollis Cline	**Zachary Mainen**
Josh Dubnau	**Senthil K. Muthuswamy**
Shiv Grewal	**Marja Timmermans**
Gregory Hannon	**Tim Tully**
Eli Hatchwell	**Linda Van Aelst**
Leemor Joshua-Tor	**Anthony Zador**

FALL COURSE CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

FUNDED IN PART BY **The Beckman Foundation**

INSTRUCTORS **Gregory Hannon (Lead)**
Hollis Cline
Winship Herr
Leemor Joshua-Tor
Senthil K. Muthuswamy
Arne Stenlund

GUEST LECTURERS **Alexander A.F. Gann**
Ira M. Hall
Nouria Hernandez
David Jackson
Adrian R. Krainer
Scott Lowe
Zachary Mainen
Robert Martienssen
Alea A. Mills
Michael P. Myers
Bruce Stillman
William Tansey
Marja Timmermans
Nicholas Tonks
Tim Tully

VISITING LECTURER **Michael Hengartner,** University of Zurich

A fundamental aspect of earning the Ph.D. is training in the pursuit of knowledge. In this core course, which forms the heart of the Fall Course curriculum, students (1) acquired a broad base of knowledge in the biological sciences, (2) learned the scientific method, and (3) learned how to think critically about biological concepts. This course consisted of 12 weekly segments, each of which had a different theme. Each week, students read an assigned set of research articles (generally four articles) and provided written answers to a problem set that guided them through two (or, occasionally, one) of the articles. Twice weekly, students attended lectures related to the week's topic, which included concepts and experimental methods. During the week, the students met to discuss the assigned papers not covered by the problem set among themselves. At the end of each weekly segment, the students submitted their problem sets and spent the evening discussing with faculty the articles not covered by the problem set. The course culminated in the 13th week with a final exam. Studying for the final exam gave the students the opportunity to synthesize and integrate what they had learned over the course of the fall term. The weekly topics were:

Week 1 Genetics
Week 2 Macromolecular Structure
Week 3 Transcriptional regulation
Week 4 Chromosome structure and function
Week 5 Post-transcriptional regulation
Week 6 Signal transduction
Week 7 Cell division cycle
Week 8 Mobile genetic elements
Week 9 Cancer genes
Week 10 Cell-cell communication
Week 11 Development
Week 12 Neurobiology
Week 13 Final Exam

The Darrell Core Course on Scientific Exposition and Ethics

FUNDED IN PART BY **The Beckman Foundation**
The John P. and Rita M. Cleary Visiting Lectureship
The Seraph Foundation Visiting Lectureship
The Susan T. and Charles E. Harris Visiting Lectureship

INSTRUCTORS **William Tansey (Lead)**
Adrian R. Krainer
Jan A. Witkowski

GUEST LECTURERS **Terri Grodzicker**
Winship Herr

VISITING LECTURERS **Richard Burian, Ph.D.,**Virginia Polytechnic Institute and State University
Robert P. Charrow, Esq., Greenberg Traurig LLP
John Doll, United States Patent and Trade Office
Richard Harris, National Public Radio
Philip Reilly, J.D., M.D., CEO of Interleukin Genetics

This core course offered instruction about the fundamental elements of scientific exposition—writing skills and public speaking—and ethics. The ability to communicate effectively and to appreciate the intricacies of ethical issues are essential skills for biologists; both subjects were taught in a series of example-based lectures and discussion groups. This year, the course continued a novel format established in 2000 in which the course was organized around the scientific process, starting with how the ideas for an experiment develop and covering execution of the experiment, presentation of the results at seminars and in publication, funding, and the implications of the experimental results on scientists and society. As a part of learning how to make oral presentations, together with the instructors, the students also critiqued formal seminar presentations at the Laboratory. A primary objective of the course was for students to consider exposition and ethics as an integral part of scientific research.

Research Topics

ARRANGED BY **Lilian Gann and Dawn Meehan**

This core course provided students with an in-depth introduction to the fields of research that the Laboratory scientists investigate. Students and faculty attended a weekly Research Topics seminar, at which faculty members presented their current research topics and methods of investigation each Wednesday evening over dinner. The students learned how to approach important problems in biology. These seminars, together with the annual fall Laboratory In-House symposium, provided students with a basis for selecting laboratories in which to do rotations. The weekly speakers were:

Week 1 Eli Hatchwell, W. Richard McCombie, Gregory Hannon
Week 2 Rui-Ming Xu, Linda Van Aelst, Dmitri Chklovskii
Week 3 Nouria Hernandez, William Tansey, Scott Lowe

Week 4	Winship Herr, Senthil K. Muthuswamy
Week 5	Carlos Brody, Lincoln Stein, Michael Q. Zhang
Week 6	Grigori Enikolopov, Leemor Joshua-Tor, Michael P. Myers
Week 7	Zachary Mainen, Josh Dubnau, Roberto Malinow
Week 8	Partha Mitra, Alexei Koulakov, Anthony Zador, Nicholas Tonks
Week 9	Bruce Stillman, Arne Stenlund, Jacek Skowronski, Adrian R. Krainer
Week 10	Robert Martienssen, Hollis Cline, Andrew Neuwald
Week 11	Michael Wigler, Alea Mills, Tatsuya Hirano
Week 12	David L. Spector, Masaaki Hamaguchi, Karel Svoboda
Week 13	David Jackson, Marja Timmermans, Robert Lucito
Week 14	Tim Tully, Yi Zhong, Z. Josh Huang
Week 15	Jerry Yin, Vivek Mittal, Yuri Lazebnik

SPECIALIZED DISCIPLINES COURSES

Cellular Structure and Function

FUNDED IN PART BY	**The Mary D. Lindsay Lectureship** **The Sigi Ziering Lectureship** **The Martha F. Gerry Visiting Lectureship**
INSTRUCTORS	**David L. Spector** **David Helfman** **Linda Van Aelst**
GUEST LECTURERS	**Tatsuya Hirano** **Yuri Lazebnik**
VISITING LECTURERS	**Fred Maxfield,** Weill Medical College **Graham Warren,** Yale University

With the complete set of instructions available for many organisms—i.e., their genome sequence—there is now an increasing emphasis on understanding the function of the gene products. This understanding will require an increasing appreciation of the structure and function of the cell. This course provided a basic overview of the structural and functional organization of cells with particular emphasis on cellular compartmentalization and communication. In addition, it provided insight into the basic toolbox of the cell biologist of the twenty-first century.

Genetics

FUNDED IN PART BY **The Edward H. and Martha F. Gerry Lectureship**
The Pfizer Lectureship
The George B. Rathmann Lectureship
The Edward H. Gerry Visiting Lectureship

INSTRUCTORS **Tim Tully**
Josh Dubnau
Lincoln Stein

VISITING LECTURER **Bambos Kyriacou,** University of Leicester, UK

The human genome sequence and continued advances in molecular biological techniques have initiated a paradigm shift in the biological sciences, from phenomenological description to genetic perspective. Genes now can be manipulated in experiments, permitting interventionist studies of their roles in various aspects of biological function. These experimental data were integrated "vertically" to understand how causal models of gene function are inferred across various levels of biological organization and lead to molecular mechanisms. Data were also integrated "horizontally" to understand how genetic pathways have been conserved evolutionarily as variations on a theme.

This course placed classical organismal genetics into the context of modern molecular biology and genomics. History, perspective, and technique were described around four levels of analysis: phenotype, genotype, variation, and genome. How do gene mutations disrupt phenotypic processes? How are complex traits genetically dissected into functional components? What concepts and techniques are used to organize genes into pathways and networks? How are genes mapped, cloned, and engineered to identify functional domains of proteins? What gene variation exists in natural populations? What are the functional consequences of gene variation? How are they detected? How are genomes organized and coordinately regulated? How can genomic information be cataloged, organized, and mined? These questions and concepts were fleshed out using examples from the literature.

Systems Neuroscience

FUNDED IN PART BY **The George W. Cutting Lectureship**
The Klingenstein Lectureship
The William Stamps Farrish Lectureship

INSTRUCTORS **Zachary Mainen**
Carlos D. Brody
Dmitri Chklovskii

GUEST LECTURERS **Hollis Cline**
Roberto Malinow
Anthony Zador

Cognition and behavior arise from complex interactions between billions of neurons. This systems neuroscience course examined fundamental properties of neurons, including synaptic transmission and plasticity, and how in higher-order organization they give rise to brain functions such as visual perception.

UNDERGRADUATE RESEARCH PROGRAM

Program Director: Leemor Joshua-Tor
Associate Program Director: Gregory Hannon
Program Administrator: Jane Reader

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time faculty members. The program was initiated in 1959. Since that year, 629 students have participated in the course and many have gone on to productive careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology; (2) an increased awareness of experimental approaches to science; (3) a deeper understanding of the major issues in the fields of biochemistry, genetics, molecular, cellular and structural biology, neuroscience, and genomics; and (4) a personal acquaintance with research, research workers, and centers for study.

During the program, the students are housed together on the Laboratory grounds. Nearly all of the students arrive at the same time and share the entire experience. For programmatic reasons, we limit the number of students to 25. In this manner, we ensure a cohesive program with substantial scientific and

Left to right: *(Front row)* Emily Anderson, Catherine Del Vecchio, Daniel Jones, Chris Brown, Shraddha Pai, Jessica Cardenas-Navia, Gabriel Orebi Gann, John McIntyre, Christine Wu, Maria Zhadina, Margaret Wat.
(Middle row) Nicholas Manicke, Jonathan Schneiderman, John Wallach, Peter Slomiany, Lieven van der Veken, Mollie Biewald, Gediminas Luksys, Galen Collins, Keisha John, Michael Minder, Rafal Klajn.
(Back row) Henry Lin, Nina Marinsek. Missing: Rittik Chaudhuri.

social interactions among the students. The students are required, at the beginning of the program, to present to their peers a concise oral description of the background and the design of the experiments they will be performing. At the end of the 10-week program, the undergraduates present a 20–30-minute seminar describing the background, design, and results of their experiments during the course of a two-day undergraduate symposium. During their stay, the participants attend a series of Faculty Talks given by both young and established scientists at the Laboratory. These seminars are attended by only the students and Program Directors to assure that the seminars remain at a level appropriate for the undergraduates. It also encourages questions in an informal and comfortable setting. In addition to scientific discussion, these presentations cover important issues such as personal experiences and choices that led the scientists to their current area of research and position. The following students, selected from 478 applicants, took part in the 2003 program:

Emily Anderson, Grinnell College
Advisor: **Dick McCombie**
Sponsor: National Science Foundation
Gene prediction: An assessment of tools.

Mollie Biewald, Columbia University
Advisor: **Josh Dubnau**
Sponsor: National Science Foundation
Oskar-GFP: In vivo imaging of RNA transport during memory.

Christopher Brown, Clemson University
Advisor: **Rob Lucito**
Sponsor: National Science Foundation
Detecting gene copy number changes in ovarian cancer.

Jessica Cardenas-Navia, Yale University
Advisor: **Yuri Lazebnik**
Sponsor: National Science Foundation
Development and implementation of a cell fusion tracking assay.

Rittik Chaudhuri, Duke University
Advisor: **David Jackson**
Sponsor: National Science Foundation
Potential plasmodesmatal receptors and an associated trafficking protein in *Arabidopsis*.

Galen Collins, Wabash College
Advisor: **Marja Timmermans**
Sponsor: Von Stade Foundation
Asymmetric leaves1: Regulation of stem cell differentiation.

Catherine Del Vecchio, Bowdoin College
Advisor: **Bill Tansey**
Sponsor: National Science Foundation
An investigation into mediator factor Med8 and its potential role in ubiquitin-mediated proteolysis.

Gabriel Orebi Gann, Cambridge University
Advisor: **Dmitri Chklovskii**
Sponsor: Burroughs Wellcome
Connectivity and interaction strength of paired neurons.

Keisha John, University of Maryland
Advisor: **Holly Cline**
Sponsor: Bliss Fund
Localization of RNAi machinery.

Daniel Jones, Pomona College
Advisor: **Josh Huang**
Sponsor: Jephson Educational Trust
Characterization of GABAergic interneuron connectivity in neocortex.

Rafal Klajn, University of Warsaw
Advisor: **Leemor Joshua-Tor**
Sponsor: Read Fund
Towards the crystal structure of BPV protein E2.

Henry Lin, Harvard College
Advisor: **Michael Zhang**
Sponsor: Garfield Fund
Comparative genome analyses.

Gediminas Luksys, Intl. University Bremen
Advisor: **Tony Zador**
Sponsor: Glass Fund
Psychophysical approaches in solving the cocktail party problem.

Nicholas Manicke, University of Evansville
Advisor: **Alea Mills**
Sponsor: National Science Foundation
Investigating the role of *p63* in the skin.

Nina Marinsek, Cambridge University
Advisor: **Rob Martienssen**
Sponsor: Libby Fund
Role of RNAi in chromatin modification and its interaction with DNA methylation.

John McIntyre, National University Ireland
Advisor: **Senthil Muthuswamy**
Sponsor: Shakespeare Fund
Gene silencing and growth control in 3D epithelial cells.

C. Michael Minder, University of North Carolina
Advisor: **Rui-Ming Xu**
Sponsor: National Science Foundation
Exploring the exon junction complex.

Shraddha Pai, University of Waterloo
Advisor: **Lincoln Stein**
Sponsor: Olney Fund
Chemoreceptor genes in *C. elegans* and *C. briggsae*.

Jonathan Schneiderman, Tel Aviv University
Advisor: **Greg Hannon**
Sponsor: Burroughs Wellcome
A species at RISC: Characterizing the recruitment of small interfering RNA in the RNAi pathway.

Peter Slomiany, Connecticut College
Advisor: **Eli Hatchwell**
Sponsor: Burroughs Wellcome
Mapping a microdeletion using a myriad of methods, including microarrays and polymorphisms.

Lieven Van der Veken, Leuven Catholic University
Advisor: **Linda Van Aelst**
Sponsor: Emmanuel Ax Fund
Oligophrenin, a study of interactions.

John Wallach, Massachusetts Institute of Technology
Advisor: **Karel Svoboda**
Sponsor: Jephson Educational Trust
Role of neural actin-binding protein in dendritic spines morphogenesis.

Margaret Wat, Duke University
Advisor: **Masaaki Hamaguchi**
Sponsor: Burroughs Wellcome
RNAi knockdown of *DBC2*.

Christine Wu, University of California, Berkeley
Advisor: **Winship Herr**
Sponsor: Jephson Educational Trust
Investigating the role of HCF-1 in mouse F9 cell differentiation.

Maria Zhadina, Brandeis University
Advisor: **David Helfman**
Sponsor: Burroughs Wellcome
Characterization of *p21* function in cell motility.

PARTNERS FOR THE FUTURE

Program Director: Yuri Lazebnik

Program Administrator: Lynn Hardin

The Partners for the Future program, established in 1990, brings Long Island high school students into Cold Spring Harbor laboratories and gives them a taste of the real world of biomedical research. The program is open to all Long Island high school students entering their senior year; each high school science chairperson may nominate three students from his or her school during their junior year. Twelve student semi-finalists are interviewed by Laboratory scientists and the six or more winners go on to spend a minimum of ten hours per week, September through March of their senior year, doing original research under the watchful eye of a scientist mentor. At the conclusion, the winning students give oral presentations of their research projects to an enthusiastic audience of the students' scientific mentors, Lab administrators, parents, and teachers. While the students learn a great deal about molecular biology and state-of-the-art research techniques, the main advantage of the program is in exposing the students to day-to-day life in a working lab. Debunking the mythical scientist-in-a-lab-coat image, the students are introduced to a world of relatively young scientists and their interactive support staff in a relaxed, problem-solving atmosphere. The 2003–2004 Partners for the Future are:

Partner	High School	CSHL Mentor	Laboratory
Jeremy Allen	Roslyn High School	Diane Esposito	Mike Wigler
Marissa Anto	St. Anthony's High School	Frances Hannan	Yi Zhong
Molly Fox	Friends Academy	Yalin Wang	Yi Zhong
Matthew Golub	Cold Spring Harbor High School	Anindya Bagchi	Alea Mills
Emily Greenberg	Island Trees High School	Masaaki Hamaguchi	
Sean Mehra	Jericho High School	Eli Hatchwell	
Melissa Teixeira	Syosset High School	Michael Regulski	Tim Tully

(*From left to right*) Melissa Teixeira, Molly Fox, Matthew Golub, Emily Greenberg, and Sean Mehra. (Missing) Marissa Anto and Jeremy Allen.

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and secondary school students the opportunity to acquire a greater knowledge and understanding of their environment. Through a series of specialized field courses, younger students can engage in introductory programs such as *Nature Bugs*, *Nature Detectives*, and *Nature Discovery* and older students can enroll in more advanced programs such as *Marine Biology*.

During the summer of 2003, 286 students participated in 26 courses within the program. The classes were held outdoors, weather permitting, at the Southdown School. The Laboratory has equipped and maintains classroom and laboratory facilities at this location. It is used as a base for the students' exploration of the local environment. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, the Nature Conservancy, the Cold Spring Harbor Fish Hatchery and Aquarium, as well as other local preserves and sanctuaries.

In addition to the three, two-week sessions, the *Adventure Education* course met on July 25, 2003, for a six-mile canoe trip on the Nissequogue River in Smithtown to navigate and explore the waters of Long Island. The course emphasizes the plant and animal life indigenous to the area as well as historic points of interest.

This year, we were able to bring back *Nature Photography*. The course focuses on taking pictures out in the field as well as in the studio and allow students to experiment with different photographic techniques. A darkroom is set up for students to develop and print their own black and white film.

PROGRAM DIRECTOR: William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR: Sharon Bense, Cold Spring Harbor Laboratory

INSTRUCTORS: **Jessica Badalucco,** B.S. in Biology, Adelphi University
Amy Friedank, B.S. in Marine Science, Long Island University, Southampton College
Jimmie Hamilton, Pratt Institute, Graphic Design/Photography
Ann Marie LaRuffa, B.A in Natural Science, Adelphi University
Michael Zarzicki, B.A. in English, Adelphi University

COURSES

Nature Bugs (Kindergarten): Exploration, games, stories, and dramatics are used to introduce the young child to the variety of natural habitats.

Nature Detectives (Grades 1–2): An introductory course in nature study, stressing interrelationships between plants and animals. A variety of habitats are explored.

Nature Discovery (Grades 1–2): Students continue their discovery of nature through activities and concepts.

Ecology Explorers (Grades 3–4): Natural communities, food webs, and the succession of communities are studied. Students study the diversity of plant and animal forms native to the Cold Spring Harbor area.

Pebble Pups (Grades 3–4): Elementary geology for students interested in making a basic study of rocks and minerals available on Long Island. Each student completes a rock and mineral collection. Dinosaurs and fossils are featured themes. Some of the highlights of this course include field trips to local museums.

Frogs, Flippers, and Fins (Grades 3–4): Program designed for younger students as an introduction to aquatic ecosystems. Fresh water and marine habitats are explored.

Seashore Life (Grades 5–7): Children examine plant and animal life found below the tidemark. Fish, marine worms, algae, shellfish, beach plants, and shore birds are studied.

Freshwater Life (Grades 5–7): Study of the vertebrate, invertebrate, and plant life found in area bogs, ponds, lakes, and streams.

Nature Photography (Grades 5–7): Students take pictures and use the darkroom to learn techniques of printing and developing. Photographic techniques relating to nature photography are emphasized.

Adventure Education (Grade 6–10): This course is a six-mile canoe trip up and down the Nissequogue River exploring the flora and fauna of the waterway.

Marine Biology (grades 8–10): This course offers a more sophisticated study of plants and animals native to the inner and outer harbors and provides field trips, dissection, use of the microscope, and laboratory experiments.

COLD SPRING HARBOR LABORATORY MEETINGS AND COURSES

ACADEMIC AFFAIRS

The academic program of meetings and courses represents a diversified year-round effort. It includes advanced laboratory courses; summer lecture and workshop courses held at the Laboratory's Banbury Center; large meetings and winter biotechnology conferences held in Grace Auditorium; and short bioinformatics courses that take place at the Woodbury campus. Scientists attending these events range from graduate students and postdoctoral fellows to senior faculty.

In 2003, 27 laboratory and lecture courses were held. These covered a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim of the courses remains to teach students the latest advances in technologies and concepts that can be immediately applied to their own research. Courses are always being evaluated and updated to include the latest concepts and approaches. For example, the course on *Advanced Techniques in Molecular Neuroscience* (formerly *Advanced Cloning of Neural Genes*) has taken advantage of sophisticated in vivo methods to affect and analyze gene function in neural cells. The course proved to be immensely popular and was heavily oversubscribed. In plant molecular biology, *Arabidopsis* has become an extremely useful model system. However, application of molecular genetic techniques to other plant systems has produced much valuable information. Thus, the *Arabidopsis Molecular Genetics* course has added significant work on other plants and will continue to emphasize this new direction.

Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor. Their excellence and dedication make the course program work so well. The full program of 2003 courses and instructors follows this report.

Grants from a variety of sources support the courses, including multiple awards from the National Institutes of Health and the National Science Foundation. We also have a valuable large education grant from the Howard Hughes Medical Institute for the support of neurobiology courses and new courses, as well as a neurobiology course grant from the Esther and Joseph A. Klingenstein Foundation. The courses also depend on equipment and reagents that are loaned or donated by a large number of companies. These are invaluable for making it possible to keep up with the latest technologies.

The Laboratory held 19 meetings this year, which brought together more than 6000 scientists from around the world to discuss their latest research. A prime feature of the meetings is that there are very few invited speakers. Meetings organizers select talks from abstracts that are submitted. This format ensures that the latest findings will be presented and that young scientists will have the chance to describe their work.

The meeting season started with a special conference on *The Biology of DNA*, held to celebrate the 50th anniversary of the double helix. As often happens, many meetings were oversubscribed. These included *Retroviruses* and *Mechanisms of Eukaryotic Transcription* as well as the annual Symposium, which was held on the topic of *The Genome of* Homo sapiens. Many of the meetings have become essential for those in the field and are held on a biannual basis. Partial support for individual meetings is provided by grants from the National Institutes of Health, National Science Foundation, Department of Energy, foundations, and companies. Core support for the meetings program is provided by the Laboratory's Corporate Benefactors, Sponsors, Affiliates, and Contributors.

The Symposium continues to be central to the meetings program. This year's meeting on *The Genome of* Homo sapiens addressed many aspects of genome science and featured 79 talks and 178 poster presentations. Opening night speakers included Jane Rogers, David Page, David Botstein, and David Cox, and Maynard Olson gave the summary. Clare Fraser presented the Reginald Harris lecture on "Microbial Genome Sequencing: Insights into Physiology, Infectious Disease, and Evolution." Francis Collins gave the Dorcas Cummings lecture on "The Human Genome Project: Where Do We Go from Here?" to a mixed audience of scientists and lay friends and neighbors of the laboratory.

The success of the very large number of meetings and courses is also due to the skilled work of many Cold Spring staff and faculty who contribute their expertise, efforts, and good humor to the program. Challenges in 2003 included the extra work associated with the 50th anniversary of the double helix, the threat of SARS, and two meetings that coincided, respectively, with the great August blackout and a major December snowstorm.

Terri Grodzicker
Assistant Director for Academic Affairs

David Stewart
Executive Director, Meetings and Courses

68TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

The Genome of *Homo sapiens*

May 28–June 2 536 participants

ARRANGED BY **Jane Rogers,** Wellcome Trust Sanger Institute
Edward Rubin, DOE Joint Genome Institute
Bruce Stillman, Cold Spring Harbor Laboratory
David Stewart, Cold Spring Harbor Laboratory

In the history of biology throughout most of the 20th century, the Cold Spring Harbor Symposia on Quantitative Biology have served an almost unique purpose by every summer bringing together groups of the world's leading scientists irrespective of race or nationality, to discuss and debate particular topics at times when those fields are making rapid progress. At each Symposium, scientific advances in the chosen field were celebrated not only among attending participants, but also through publication of the conference proceedings, to a wider audience of researchers.

The 1986 Cold Spring Harbor Symposium on "Molecular Biology of *Homo sapiens*" witnessed the first public discussion of the proposal to sequence the entire human genome and marked the start of the global initiative leading to the current Human Genome Project. For 15 years since, genome scientists have been gathering annually at Cold Spring Harbor for four days in early May to discuss progress in mapping, sequencing, and analyzing genomic information. In 2001, after discussion with leading genome scientists including Francis Collins, current head of the Human Genome Project, Cold Spring Harbor Laboratory decided that in 2003 the annual genome meeting and the annual Symposium should be merged into a single meeting. The 2003 Symposium was therefore chosen to focus on "The Genome of *Homo sapiens*," aimed to coincide with the completion of the finished sequence of the human genome, the spectacular culmination of a vast amount of work in major genome centers and countless laboratories around the world. Furthermore, this Symposium also marked the 50th anniversary of the discovery of the double-helical structure of DNA in 1953. While this anniversary was celebrated through multiple events in the United States, the United Kingdom, France, and Australia, the Cold Spring Harbor Symposium was almost unique in combining celebrations of that anniversary together with the completion of the human genome.

The 2003 Cold Spring Harbor Symposium witnessed extraordinary advances in the annotation of the human genome, providing revealing molecular insights about the origins of *Homo sapiens*, the structure and dynamics of our genome, the global regulation of gene expression, and the underlying genetic component of many human diseases. Held over a six-day period at the beginning of June, the Symposium hosted 536 scientists from more than 30 countries. Topics at the Symposium included The Human Genome Sequence; Genome Structure; Genome Evolution; Comparative Genomics and Human Evolution; Genetic Polymorphisms and Disease; Functional Genomics and Proteomics; Co-Evolution of Human Pathogens; and Bioinformatics.

The Symposium program included 80 oral presentations, including keynote talks given by Jane Rogers, David Botstein, David Cox, David Page, and Claire Fraser; a fascinating round-table panel discussion on Genes, Genomes, and Society; as well as 180 posters in two sessions. The hour-long Dorcas Cummings lecture to the meeting and the local community was given by Francis Collins and attracted over 800 people in total. At the conclusion of the Symposium, Maynard Olson of Washington University provided a masterly

F. Collins, Dorcas Cummings Lecture

Summary of the current state of the field (delivered to a spell-bound audience in near darkness because of a power outage).

Essential funds to run this meeting were obtained from the National Genome Research Institute and the National Cancer Institute (branches of the National Institutes of Health) and the U.S. Department of Energy. In addition, financial help from the corporate benefactors, sponsors, affiliates, and contributors of our meetings program is essential for these Symposia to remain a success and we are most grateful for their continued support. *Corporate Benefactors:* Amgen, Inc.; Aventis Pharma AG; Bristol-Myers Squibb Company; Eli Lilly and Company; GlaxoSmithKline; Novartis Pharma AG; Pfizer Inc. *Corporate Sponsors:* Applied Biosystems; AstraZeneca; BioVentures, Inc.; Cogene BioTech Ventures, Ltd.; Diagnostic Products Corporation; Forest Laboratories, Inc.; Genentech, Inc.; Hoffmann-La Roche Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Kyowa Hakko Kogyo Co., Ltd.; Lexicon Genetics, Inc.; Merck Research Laboratories; New England BioLabs, Inc.; OSI Pharmaceuticals, Inc.; Pall Corporation; Schering-Plough Research Institute; Wyeth Genetics Institute. *Plant Corporate Associates:* MeadWestvaco Corporation; Monsanto Company; Pioneer Hi-Bred International, Inc. *Corporate Affiliates:* Affymetrix, Inc. *Corporate Contributors:* Biogen, Inc.; ImmunoRx, Inc.; KeyGene. *Foundations:* Albert B. Sabin Vaccine Institute, Inc.

B. Vincent, P. Callisan, J. Myers

J. Bertranpetit, D. Botstein

P. Good, G. Rubin

PROGRAM

Introduction
J.D. Watson, *Cold Spring Harbor Laboratory*

Human Genome Sequence
Chairperson: E. Ostrander, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

Genome Structure and Evolution
Chairperson: E. Lander, *Whitehead Institute/MIT Center for Genome Research, Cambridge, Massachusetts*

Genomics of Nonhuman Species
Chairperson: E. Rubin, *Joint Genome Institute, Berkeley, California*

Forum on Genes, Genomes, and Society
Chairperson: J. Witkowski, *Cold Spring Harbor Laboratory*

Reginald G. Harris Lecture: Bioinformatics/Genome Annotation
Chairperson: J. Rogers, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Human Genetic Variation
Chairperson: R. Gibbs, *Baylor College of Medicine, Houston, Texas*

Of Microbes and Men
Chairperson: E. Green, *NHGRI, National Institutes of Health, Bethesda, Maryland*

Comparative Functional Genomics
Chairperson: L. Stubbs, *Lawrence Livermore National Laboratory, California*

Genetic Variation and Disease
Chairperson: K. Frazer, *Perlegen Sciences, Mountain View, California*

Comparing Genomes
Chairperson: R. Waterston, *Washington University School of Medicine, St. Louis, Missouri*

Dorcas Cummings Lecture: The Human Genome Project: Where Do We Go from Here?
Speaker: Francis Collins, *National Human Genome Research Institute*

Human Evolution
Chairperson: A. Chakravarti, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Genome Biology
Chairperson: S. Lewis, *University of California, Berkeley*

Summary
Maynard Olson, *University of Washington, Seattle*

E. Lander, F. Collins, J. Watson

R. Waterston, L. Hood, B. Stillman

MEETINGS

The Biology of DNA

February 26–March 2 310 participants

ARRANGED BY **David Stewart,** Cold Spring Harbor Laboratory
Jan Witkowski, Cold Spring Harbor Laboratory

The Double Helix 50th Anniversary celebration at Cold Spring Harbor Laboratory took the form of a four-day scientific conference, "The Biology of DNA," that highlighted the past, present, and future of DNA science. The meeting opened with introductory remarks from Jan Witkowski, followed by Bruce Stillman's introduction of Francis Crick's taped remarks on the excitement surrounding the double helix discovery.

2002 Nobel laureate Sydney Brenner spoke after Francis, explaining why the DNA structure revolutionized biology. According to Sydney, it was not the double helix itself, but its immediate implications that made—and still make—the structure so important. These implications were examined throughout the course of the meeting including 1959 Nobel laureate Arthur Kornberg's talk on how DNA might be replicated; 1968 Nobel laureate Marshall Nirenberg's thoughts on how protein sequences are encoded by DNA sequences, how genes are shuffled during sexual reproduction, how mutations occur and are corrected, how the large-scale structure of DNA impacts its function, human evolution, and even the origin of life. 1980 Nobel laureate Walter Gilbert, Sir Alec Jeffreys, Eric Lander, and Francis Collins were also on hand to offer choice comments from their perspectives.

Overall, "The Biology of DNA" was an inspiring celebration of the overwhelmingly positive outcomes for humanity and human health that have stemmed from Watson and Crick's discovery 50 years ago and will reverberate for at least another 50. The meeting is available as a video archive at www.cshl.edu/leadingstrand/.

Major sponsorship for this meeting was provided by Biogen, Inc. and Virgin Atlantic Airways. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

P. Schimmel, P. Sharp, H. Smith

M. Meselson, S. Brenner, S. Tingey, A.-M. Skalka

A. Rich

PROGRAM

Introduction
B. Stillman, *Cold Spring Harbor Laboratory*

Replication
Chairpersons: B. Alberts, *National Academy of Sciences, Washington, D.C.*; T. Grodzicker, *Cold Spring Harbor Laboratory*

Topology and Chromosome Dynamics
Chairperson: A.-M. Skalka, *Fox Chase Cancer Center, Philadelphia, Pennsylvania*

Discussion: Biologists' Responsibilities in a Time of Crisis
Chairperson: M. Meselson, *Harvard University, Cambridge, Massachusetts*

Recombination
Chairpersons: A. Pardee, *Dana-Farber Cancer Institute, Boston, Massachusetts;* A. Chakravarti, *Johns Hopkins Hospital, Baltimore, Maryland*

Mutagenesis and Repair
Chairperson: M. Radman, *INSERM, Paris, France*

Genetic Code and the Origins of Life
Chairpersons: C. Debouck, *GlaxoSmithKline, King of Prussia, Pennsylvania;* T. Caskey, *Cogene Biotech Ventures, Houston, Texas*

Remarkable Things That Could Not Have Been Noticed
Chairpersons: F. Perler, *New England Biolabs, Beverly, Massachusetts;* N. Zinder, *The Rockefeller University, New York*

Remarks
W. Szybalski, *University of Wisconsin, Madison*

DNA: A Genome-scale Perspective
Chairpersons: S. Lewis, *University of California, Berkeley;* H. Smith, *Celera Genomics, Rockville, Maryland*

Closing Remarks
J.D. Watson, *Cold Spring Harbor Laboratory*

E. Witkin, E. Friedberg

W. Szybalski

N. Zinder, G. Walker, M. Capecchi

D. Micklos, A. Jeffreys, M. Radman

Systems Biology: Genomic Approaches to Transcriptional Regulation

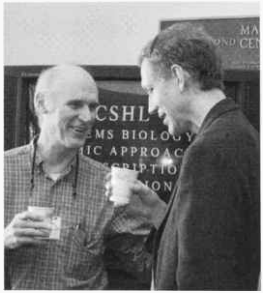
March 6–9 203 participants

ARRANGED BY **Philip Benfey,** Duke University
Susan Celniker, Lawrence Berkeley National Laboratory
Stephen Small, New York University
Michael Zhang, Cold Spring Harbor Laboratory

Deciphering the regulatory codes that control which genes are expressed in different cells is a major challenge in the postgenomic era. This problem has been traditionally approached by experimental biologists, who have identified several hundred DNA elements that exhibit enhancer and insulator activities. These elements contain specific binding sites for proteins that mediate or prevent transcriptional activation. More recently, the completion of several genome sequences and compilations of genome-wide expression data have spawned a series of computational approaches aimed at solving this problem. This meeting focused on recent advances from both approaches, and provided a forum for cross-disciplinary interactions. Sessions outlined recent progress in understanding DNA-protein interactions and new computational approaches for predicting functional motifs in genomic sequences. Other sessions were dedicated to mining regulatory information by examining the regulatory regions of coexpressed genes or by analyzing sequence conservation between evolutionary taxa. A final session on emerging techniques closed the meeting. It was clear that rapid progress has been made in the last few years and that further interactions between experimental and computational biologists will speed our understanding of the genome-wide mechanisms that control gene expression. A planning session was held, and a steering committee was appointed to foster similar interactions in the future.

This meeting was funded in part by the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

M. Caudy, V. Iyer



C. Lawrence, P. Benfey

R. Weinzierl, M. Frith

PROGRAM

Keynote Address: Global Analysis of Conserved Genetic Pathways and Molecular Machines
S. Kim, *Stanford University Medical Center, California*

TF-DNA Interactions
Chairpersons: S. Small, *New York University, New York;* M. Levine, *University of California, Berkeley*

Computational Approaches to Identifying CREs I
Chairpersons: M. Eisen, *Lawrence Berkeley National Laboratory, California;* G. Stormo, *Washington University School of Medicine, St. Louis, Missouri*

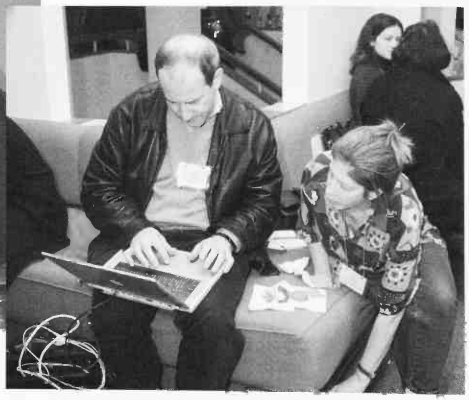
Comparative Genomics
Chairpersons: S. Celniker, *Lawrence Berkeley National Laboratory, California;* E. Rubin, *DOE Joint Genome Institute, Berkeley, California*

Computational Approaches to Identifying CREs II
Chairpersons: M. Zhang, *Cold Spring Harbor Laboratory;* E. Siggia, *The Rockefeller University, New York*

Transcriptional Network Modeling
Chairpersons: E. Davidson, *California Institute of Technology, Pasadena;* S. Leibler, *The Rockefeller University, New York*

Emerging Technologies
Chairpersons: P. Benfey, *Duke University, Durham, North Carolina;* S. Kim, *Stanford University Medical Center, California*

E. Davidson, R. Young



B. Arnow, F. Sladek

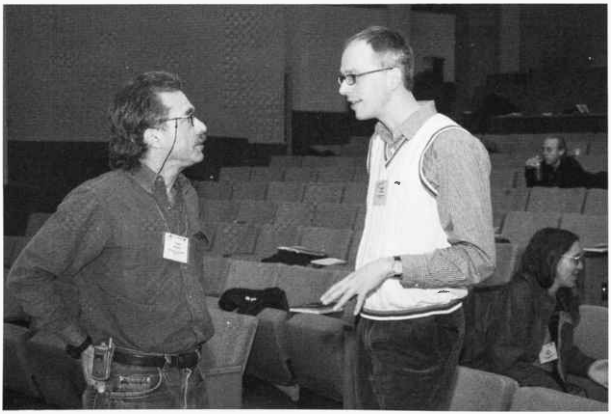
Vector Targeting Strategies for Gene Therapy

March 20–23 102 participants

ARRANGED BY **David T. Curiel,** University of Alabama, Birmingham
Wayne Marasco, Dana Farber Cancer Institute
Stephen Russell, Mayo Foundation

The paramount requirement for advancement of gene therapy is the development of vector systems possessing the capacities for efficient and cell-specific gene delivery. The achievement of these goals requires the vector system to recognize specific cell signatures. In the first regard, both nonviral and viral vectors have been engineered to address this goal. Furthermore, a variety of high-throughput methods have been advanced for identification of cell-specific markers. Investigators at this meeting provided an update of key technologies relevant to these goals. Since the last conference in 2001, progress has been noted in this field. Especially noteworthy was the progress achieved in the content of viral vector tropism modifications, targeted gene knockout and imaging technologies. This progress includes the proposed translation of novel, advanced generation vectors into the human clinical context. Additionally, the means to make targeted vectors available for clinical investigators remains a challenge. Future work will be required to define the range of cell-specific signatures of relevance in the clinical context. Nonetheless, the linkage of these relevant technologies—target definition, vector targeting, and imaging—is already yielding important advances in outcomes achievable via gene therapy methods.

This meeting was funded in part by Cell Genesys; Genzyme; Directgene, Inc.; Lexigen; Onyx Pharmaceuticals; VectorLogics, Inc., and VirRx, Inc. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.



W. Marasco, T. Trischl

PROGRAM

Introduction
Chairperson: D. Curiel, *University of Alabama, Birmingham*

Keynote Speakers
I. Weissman, *Stanford University School of Medicine, California*; E. Ruoslahti, *Burnham Institute, La Jolla, California*

Target Definition/S1 RNA
Chairpersons: J. Marks, *University of California, San Francisco;* G. Hannon, *Cold Spring Harbor Laboratory*

Vascular Targeting
Chairperson: S. Russell, *Mayo Foundation, Rochester, Minnesota*

Transcriptional Targeting/Nonviral Vectors
Chairpersons: M. Yamamoto, *University of Alabama, Birmingham;* P. Scaria, *Intradigm Corporation, Rockville, Maryland*

RNA Viruses
Chairperson: W. Marasco, *Dana-Farber Cancer Institute, Boston, Massachusetts*

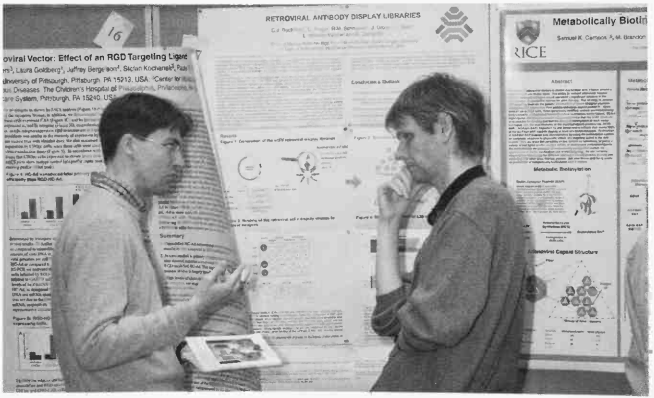
Adenoviruses
Chairperson: D. Curiel, *University of Alabama, Birmingham*

Imaging
Chairperson: S. Gambhir, *University of California, Los Angeles*

Cellular Vehicles/AAV
Chairpersons: R. Vile, *Mayo Clinic, Rochester;* M. Hallek, *University of Munich, Germany*

G. Hannon, C. Curiel

N. Mignet, M. Hitt



O. Müller, C. Buchholz

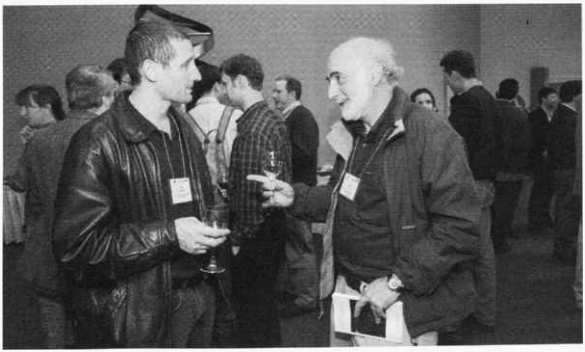
Learning and Memory

April 9–13 150 participants

ARRANGED BY **John Byrne,** University of Texas, Houston Medical School
Joseph LeDoux, New York University
Erin Schuman, California Institute of Technology

This year's meeting had a strong focus on the molecular mechanisms underlying plasticity. Two sessions highlighted local control of plasticity at the synapse. In these sessions, topics such as synaptic tagging, plasticity-dependent regulation of protein translation at the dendrite, and protein trafficking were discussed. There was a particular emphasis on regulation of the mTOR translational pathway and the ubiquitin/proeasome protein degradation pathway at the dendrite. The LTP/LTD session was largely dominated by presentations on the role of the ERK kinase and PI3 kinase signaling pathways in plasticity. In the Genetics and Behavior Session, data were presented on the role of molecules such as the phosphatases PP2B (calcineurin) and PP1, the NMDA receptor, and gastrin-releasing peptide (GRP) in learning and memory from genetically manipulated mice. Analyses or the role of the *rutabaga* gene in memory in *Drosophila* was also presented. A gene chip analysis of genes differentially expressed in the wild-type and mouse model of Alzheimer's disease following a fear-conditioning paradigm was also presented. The molecular aspect of the meeting finished up with a session on proteomics in which presentations introduced the technique and its applicability to questions surrounding analysis of synaptic proteins. In this session, other aspects of protein regulation in *Drosophila* and *Hermissenda* were also presented. There were two more systems-level sessions on consolidation and reconsolidation and on extinction. Presentations in the Consolidation/Reconsolidation session discussed topics focusing on the similarity/dissimilarity of mechanisms underlying the two phenomenona and investigated the role of different molecular pathways involved. Finally, in the Extinction session, discussion focused on the neural mechanisms of extinction and the extent to which they involve reversing the processes involved in acquisition. In addition, there was particular focus on mechanisms of enhancing the extinction of fear responses as a therapeutic strategy for phobias and post-traumatic stress syndrome.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health; and the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.



J. Dubnau, J. Byrne

PROGRAM

Local Synaptic Control I
Chairperson: K. Martin, *University of California, Los Angeles*

Local Synaptic Control II
Chairperson: J. Byrne, *University of Texas Medical School, Houston*

Genetics and Behavior
Chairperson: M. Mayford, *Scripps Research Institute, La Jolla, California*

LTP/LTD
Chairperson: J. LeDoux, *New York University*

Proteomics and Other Aspects of Protein Regulation
Chairperson: S. Grant, *University of Edinburgh, United Kingdom*

Consolidation and Reconsolidation
Chairperson: Y. Dudai, *Weizmann Institute of Science, Rehovot, Israel*

Extinction and Other Dynamics of Learning
Chairperson: M. Mauk, *University of Texas Medical School, Houston*

Hippocampus and Other Cortical Systems
Chairperson: M. Wilson, *Massachusetts Institute of Technology, Cambridge*

S. Grant, L. de Hoz, H. Husi

S. Cushman, Ellie

E. Klann, J.D. Sweatt

The Ubiquitin Family

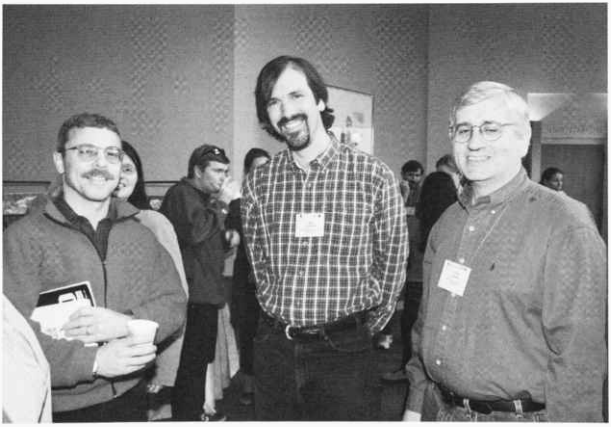
April 23–27 306 participants

ARRANGED BY **Ray DeShaies,** California Institute of Technology
Mark Hochstrasser, Yale University
Peter Howley, Harvard Medical School

This meeting represented a shift in emphasis from previous meetings in the series, formerly titled either "Biology of Proteolysis" or "Proteolysis and Biological Control." The meeting focused on the central role of ubiquitin and ubiquitin-like protein modifications in protein regulation and turnover. Research in the area of proteolysis has expanded to the point where a more defined emphasis was needed, and studies on ubiquitin and related molecules have grown exponentially in the past few years, with many exciting new findings.

The new meeting attracted more than 300 scientists who discussed the role of ubiquitin and ubiquitin-like molecules in a variety of key cellular processes. These included chromatin structure and transcription, cell cycle, signaling, apoptosis, endocytosis, membrane trafficking, and pathology. The reported experimental systems ranged from yeast to humans, and the interests of speakers varied from analysis of protein structure and enzymatic pathways to human cancer and neurodegenerative diseases. Despite such diversity, the participants commented that the meeting was focused, informative, and exciting. In summary, The Ubiquitin Family meeting was a unifying forum that is helping us to understand the key roles that ubiquitin and the related ubiquitin-like molecules have in cellular physiology and disease.

This meeting was funded in part by the National Cancer Institute, the National Institute of Aging, and the National Institute of Child Health and Human Development, branches of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.



M. Hochstrasser, R. Deshaies, P. Howley

PROGAM

Chromatin Structure, Transcription, DNA Repair
Chairpersons: M. Hochstrasser, *Yale University, New Haven, Connecticut*; W. Tansey, *Cold Spring Harbor Laboratory*

Ubiquitin Ligases and Deubiquitinating Enzymes
Chairpersons: K. Wilkinson, *Emory University School of Medicine, Atlanta, Georgia*; A. Weissman, *National Cancer Institute, Frederick, Maryland*

Ubiquitin and Ubiquitin-like Proteins
Chairpersons: F. Melchior, *Max-Planck-Institute for Biochemistry, Martinsried, Germany*; C. Pickart, *Johns Hopkins University, Baltimore, Maryland*

Protein Quality Control
Chairpersons: T. Rapoport, *Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts*; R. Hampton, *University of California, San Diego*

Cell Cycle, Signaling, Apoptosis
Chairpersons: M. Kirschner, *Harvard Medical School, Boston, Massachusetts*; T. Hunter, *Salk Institute, La Jolla, California*

Endocytosis and Membrane Trafficking
Chairpersons: L. Hicke, *Northwestern University, Evanston, Illinois*; Y. Ohsumi, *Okazaki National Research Institute, Japan*

Physiology and Disease
Chairpersons: H. Ploegh, *Harvard Medical School, Boston, Massachusetts*; R. Kopito, *Stanford University, California*

Proteasome
Chairpersons: T. Baker, *Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge*; D. Finley, *Harvard Medical School, Boston, Massachusetts*

D. Schmidt, G. Moldovan, W. Piwko

J. Feng, B. Kuhlman

K. Yamoah, S. Israeli

Telomeres and Telomerase

April 30–May 4 286 Participants

ARRANGED BY **Titia de Lange,** The Rockefeller University
Carol Greider, Johns Hopkins University School of Medicine
Vicki Lundblad, Baylor College of Medicine

The conference consisted of seven sessions of talks as well as three poster sessions. As in 1999 and 2001, the format chosen was to invite two chairs per session, who were either established scientists in the field or new independent investigators who have already made their mark. Many session chairs also gave a scientific (12 minutes) presentation. The rest of the presentations (mostly 12 minutes) were chosen from submitted abstracts, allowing as many presentations as possible. These presentations were given primarily by graduate students and postdoctoral fellows. Attendance exceeded 300 participants, a high fraction of whom presented the 118 posters and 73 talks.

The talks and posters covered all aspects of telomere and telomerase biology, including telomerase structure, enzymology, and regulation; telomere length regulation, protection, and processing of chromosome ends; the consequences of telomere dysfunction; telomere dynamics in cancer; and telomerase-independent telomere maintenance.

The scientific content was very high throughout the conference in both the talks and the posters. A large body of unpublished data was presented and extensively discussed in an open fashion. Formal and informal discussions were lively and informative. The conference was judged to be highly successful based on verbal and e-mail communications to the organizers. There is strong enthusiasm for another meeting on the same topic in 2005.

This meeting was funded in part by the National Institute on Aging, and the National Institute of Child Health and Human Development, branches of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

C. Greider, P. Wellinger

PROGRAM

Telomerase Structure and Enzymology
Chairpersons: C. Greider, *Johns Hopkins University School of Medicine, Baltimore, Maryland;* K. Freidman, *Vanderbilt University, Nashville, Tennessee*

Telomerase Recruitment and Regulation
Chairpersons: E. Blackburn, *University of California, San Francisco;* A. Lustig, *Tulane University Health Science Center, New Orleans, Louisiana*

Telomere Length Regulation
Chairpersons: S. Smith, *New York University Medical Center;* V. Zakian, *Princeton University, New Jersey*

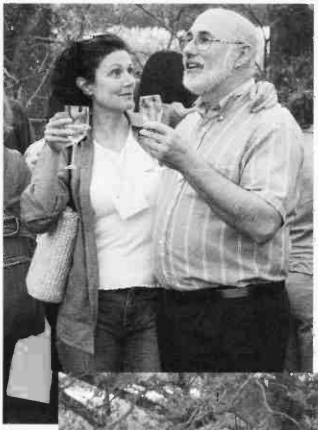
End Protection and Resection
Chairpersons: D. Wuttke, *University of Colorado, Boulder;* V. Lundblad, *Baylor College of Medicine, Houston, Texas*

Consequences of Telomere Dysfunction
Chairpersons: T. de Lange, *The Rockefeller University, New York;* S. Jackson, *University of Cambridge, United Kingdom*

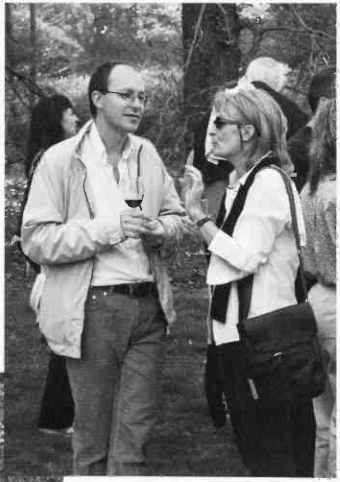
Senescence and Cancer
Chairpersons: R. DePinho, *Dana Farber Cancer Institute, Boston, Massachusetts;* R. Weinberg, *Whitehead Institute for Biomedical Research, Cambridge, Massachusetts*

Telomere and Subtelomere Chromatin
Chairpersons: J. Shay, *University of Texas Southwestern Medical Center, Dallas;* M. Biasco, *National Centre of Biotechnology, Madrid, Spain*

Telomerase-independent Telomere Maintenance
Chairpersons: R. Reddel, *Children's Medical Research Institute, Westmead, Australia;* D. Broccoli, *Fox Chase Cancer Center, Philadelphia, Pennsylvania*



T. de Lange, J. Shay



G. Parkinson, D. Rhodes

E. Louis, V. Zakian

Genome Informatics

May 7–11 308 participants

ARRANGED BY **Ewan Birney,** European Bioinformatics Institute
Suzanna Lewis, University of California, Berkeley
Lincoln Stein, Cold Spring Harbor Laboratory

The third Cold Spring Harbor Laboratory–Wellcome Trust Conference on Genome Informatics continues to highlight the latest developments in genome research and, once again, was a vital and exciting meeting, despite uncharacteristically rainy weather. This year, the conference was held for the first time at Cold Spring Harbor Laboratory, a new venue that offered new perspectives. The meeting will now be held here in upcoming alternating years. The conference followed a traditional format similar to traditional Cold Spring Harbor meetings, in that the majority of oral presentations were drawn from openly submitted abstracts.

The explosion of biological data requires a concomitant increase in the scale and sophistication of information technology. This ranges from the storage of data and their associated data models, to the design of effective algorithms to uncover nonobvious aspects of these data sets, to ontologies to concisely describe biological information, and finally to software systems to support curation, visualization, and exploration. The conference brought together some of the leading scientists in this growing field, and researchers from other large-scale information-handling disciplines were also invited to attend. Topics included Comparative Genomics, Algorithms, Large-scale Genomics, Functional Genomics, Proteomics, Databases, Knowledgebases, and Ontologies. In all, 308 participants attended, with more than 27% of delegates coming from outside North America, a highly unusual statistic for a U.S. meeting. The meeting hosted 158 scientific presentations in talks and posters.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

M. Yandell, S. Lewis, P. Carninici

PROGRAM

Keynote Address
W. Ford Doolittle, *Dalhousie University, Halifax, Nova Scotia*

Databases and Ontologies I
Chairpersons: K. Buetow, *NCI Center for Bioinformatics, Bethesda, Maryland*; M. Yandell, *University of California, Berkeley*

Databases and Ontologies II
Chairpersons: K. Buetow, *NCI Center for Bioinformatics, Bethesda, Maryland*; M. Yandell, *University of California, Berkeley*

Proteomics
Chairpersons: S. Brenner, *University of California, Berkeley*; H. Hermjakob, *European Bioinformatics Institute, Hinxton, United Kingdom*

Comparative Genomics I
Chairpersons: E. Rivas, *Washington University, St. Louis, Missouri*; S. Jones, *BC Genome Sciences Center, Vancouver, Canada*

Algorithms I
Chairpersons: R. Guigo, *Institut Municipal d'Investigacio Medica, Barcelona, Spain*; G. Myers, *University of California, Berkeley*

Functional Genomics I
Chairpersons: M. Eisen, *Lawrence Berkeley National Laboratory, California*; B.F.F. Ouellette, *University of British Columbia, Vancouver, Canada*

Large-scale Genomics I
Chairpersons: E. Stupka, *Institute of Molecular and Cell Biology, Singapore*; J. Quackenbush, *The Institute for Genomic Research, Rockville, Maryland*

Large-scale Genomics II
Chairpersons: E. Stupka, *Institute of Molecular and Cell Biology, Singapore*; J. Quackenbush, *The Institute for Genomic Research, Rockville, Maryland*

Comparative Genomics II
Chairpersons: E. Rivas, *Washington University, St. Louis, Missouri*; S. Jones, *BC Genome Sciences Center, Vancouver, Canada*

Algorithms II
Chairpersons: R. Guigo, *Institut Municipal d'Investigacio Medica, Barcelona, Spain*; G. Myers, *University of California, Berkeley*

Functional Genomics II
Chairpersons: M. Eisen, *Lawrence Berkeley National Laboratory, California*; B.F.F. Ouellette, *University of British Columbia, Vancouver, Canada*

S. Morishita, A. Schlauersbach, D. Leyfer

L. Stein, M. Ayele, J. Wortman

S. Shah, F. Ouellette, B. Butler

Protein Phosphorylation and Cell Signaling

May 14–18 228 Participants

ARRANGED BY **Sara Courtneidge,** Van Andle Research Institute
Ben Neel, Beth Israel Deaconess Medical Center
Nicholas Tonks, Cold Spring Harbor Laboratory

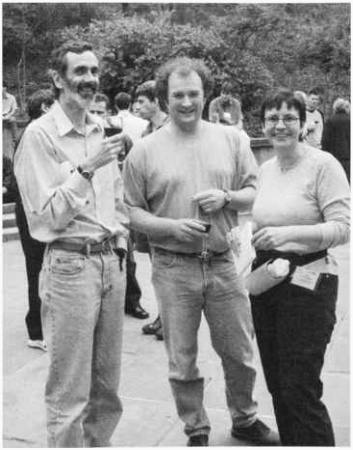
This fifth Protein Phosphorylation and Cell Signaling meeting brought together 228 scientists from the United States and abroad. Specifically focused on tyrosine phosphorylation in prior years, we expanded this meeting to include the most current work on the structure, regulation, and function of all protein kinases and protein phosphatases in biology. The meeting began with two opening Keynote Addresses by Elaine Fuchs and Phillip Sharp.

Elaine Fuchs reviewed the molecular mechanisms underlying the development and differentiation of the mammalian skin epidermis and its appendages, and how these processes go awry in various human diseases of the skin, including genetic diseases and skin cancer. Phillip Sharp reviewed progress in the field of RNA interference, in which the introduction of homologous short double-stranded RNA (dsRNA) specifically targets a gene's mature mRNA for destruction, thus creating a null or hypomorphic mutation.

The format of the meeting was designed around areas of biological interest, rather than according to particular enzymes or enzyme families. A major thrust within the pharmaceutical industry is to exploit signal transduction pathways as sources of targets for novel therapeutic strategies. The launch of Gleevec, an inhibitor of the p210 Bcr-Abl protein tyrosine kinase for the treatment of chronic myelogenous leukemia, is a spectacular example of this trend. The program thus addressed the role of protein phosphorylation in the regulation of signal transduction under normal and pathophysiologoical conditions. In addition, we highlighted recent technical advances that are facilitating the discovery process in the area of signal transduction, including the development of cell and animal model systems and recent advances in RNA interference.

The program included scientists from the United States, Europe, the Far East, and the South Pacific. Forty-nine speakers were selected to present their data in sessions that dealt with Receptor Signaling, Phosphorylation and Cancer, Signal Transduction and Control of Proliferation, Cytoskeleton and Cell Adhesion, Immune Cell Signaling, Cell Cycle and Phosphorylation and Disease. A variety of systems were described with great progress reported in genetic and biochemical approaches to the characterization of physiological functions for protein phosphorylation. In particular, exciting insights were provided into how signaling pathways may be abrogated in a variety of human disease states and to the identity of novel targets for therapeutic intervention. The meeting continues to be successful and alternates with a conference with the same format at The Salk Institute, with the result that there is an annual meeting on "Tyrosine Phosphorylation" that alternates in venue between Cold Spring Harbor and The Salk.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health; and Cell Signaling Technology, Inc. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.



J. Cooper, N. Tonks, S. Courtneidge

PROGRAM

Keynote Speakers
E. Fuchs, *Howard Hughes Medical Institute/The Rockefeller University, New York;* P. Sharp, *Massachusetts Institute of Technology, Cambridge*

Receptor Signaling
Chairperson: M. Park, *McGill University, Montréal, Canada*

Phosphorylation and Cancer
Chairperson: M. Birnbaum, *Howard Hughes Medical Institute/University of Pennsylvania, Philadelphia*

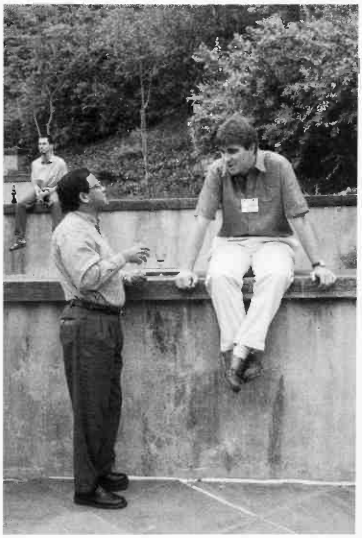
Signal Transduction and Control of Proliferation
Chairperson: H. Piwnica-Worms, *Howard Hughes Medical Institute/Washington University, St. Louis, Missouri*

Cytoskeleton and Cell Adhesion
Chairperson: M. Frame, *Beatson Institute for Cancer Research, Glasgow, United Kingdom*

Immune Cell Signaling
Chairperson: P. Schwartzberg, *NHGRI, National Institutes of Health, Bethesda, Maryland*

Cell Cycle
Chairperson: J. Cooper, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

Phosphorylation and Disease
Chairperson: M. Tremblay, *McGill University, Montréal, Canada*



B. Datta, A. Catling

W. Swiatek, E. Eide

L.A. Garulacan, T. Kline, V. Lamian

Retroviruses

May 20–25 420 participants

ARRANGED BY **Suzanne Sandmeyer,** University of California, Irvine
Wes Sundquist, University of Utah, Salt Lake City

This 28th annual Retroviruses meeting originated in 1975 as a meeting on RNA Tumor Viruses and evolved to its current focus on Retroviruses in 1993. In our view, the 28th meeting was highly successful and was enriched by two keynote talks. The first Keynote Speaker, Inder Verma, recounted the history of retroviruses and gave a thoughtful discussion of their future as vehicles for gene manipulation and gene therapy in his talk "From Reverse Transcriptase to Gene Therapy—A 30-year Journey." The second keynote speaker, Stephen Goff, discussed exciting developments in the problem of identification of host functions that participate in retrovirus replication in his talk, "Beg, Borrow or Steal—Exploitation of Host Factors in Retrovirus Replication." Both talks were very well received.

This meeting also included 315 platform and poster presentations, distributed into 13 scientific sessions. Although the focus for many years has been on individual viral functions, the focus in this year's meeting clearly shifted to the more complex role of the host in promoting and antagonizing different stages of retroviral replication. This is now possible to study because some of the cellular genes that affect retrovirus replication have been identified through high-throughput screening efforts in yeast and mammalian cells. Presentations from retrovirus and retrotransposon laboratories described new host factors involved in cellular trafficking and viral budding, viral RNA localization, viral DNA synthesis and mutagenesis, and integrase localization and activity. Particularly exciting research areas included the roles of endogenous viruses in host-species-specific restriction factors, evidence for a novel "lariat" structure for the RNA template during reverse transcription of retrotransposons and retroviruses, and the unveiling of the mechanism of the viral Vif protein and its cellular target, APOBEC15.

In other matters, the pattern of arranging posters alphabetically was continued this year, and two poster sessions followed keynote talks in the evening (one occurred in the afternoon). We thought that this format worked well and also got that feedback from others. The cuisine continues at the enjoyable level noted for last year, which was particularly fortunate this year because it rained every day! Finally, Tom Hope lightened the general atmosphere by handing out "It's the Cell" buttons to many attendees, and then presenting a (pretend) digital tracking of their movements at the Sunday morning session.

W. Sundquist, S. Goff

J. Baumann, S. Breun, J. Mbisa

This certainly enlivened the session, but the Sunday morning session itself continues to be a bit of a problem as it is difficult for people to stay on, get up, and/or be enthusiastic at this final session. Finally, we very much appreciated the help and guidance of the CSHL staff, who made the meeting easy and enjoyable to organize.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations provided core support for this meeting.

PROGRAM

Packaging, Dimerization, and Translation
Chairpersons: R. Marquet, *CNRS, Strasbourg, France*; L. Kleiman, *Jewish General Hospital, Montréal, Canada*

Assembly I
Chairpersons: J. Lingappa, *University of Washington, Seattle;* H. Göttlinger, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Assembly II
Chairpersons: W. Mothes, *Yale University School of Medicine, New Haven, Connecticut*; M. Thali, *University of Vermont, Burlington*

Envelope
Chairpersons: E. Stephens, *University of Kansas Medical Center, Kansas City*; M. Roth, *UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey*

Viral Entry
Chairpersons: J. Cunningham, *Harvard Medical School, Boston, Massachusetts;* P. Cannon, *University of Southern California, Los Angeles*

Keynote Speaker: From Reverse Transcriptase to Gene Therapy—A 30-year Journey
Inder Verma, *Salk Institute*

Pathogenesis/Host Factors I
Chairpersons: A. Engelman, *Dana-Farber Cancer Institute, Boston, Massachusetts;* F. Kirchhoff, *Universitätsklinikum Ulm, Germany*

Pathogenesis/Host Factors II
Chairpersons: D. Trono, *University of Geneva, Switzerland;* M. Palmarini, *University of Georgia, Athens*

Keynote Speaker: Beg, Borrow, or Steal—Exploitation of Host Factors in Retrovirus Replication
Stephen Goff, *Columbia University, New York*

Reverse Transcription/Recombination
Chairpersons: V. Pandey, *UMDNJ-New Jersey Medical School, Newark;* K. Musier-Forsyth, *University of Minnesota, Minneapolis*

Integration
Chairpersons: A.-M. Skalka, *Fox Chase Cancer Center, Philadelphia, Pennsylvania;* G. Kalpana, *Albert Einstein College of Medicine, Bronx, New York*

Transcription, RNA Export, and Processing
Chairpersons: T. Hope, *University of Illinois, Chicago;* A. Telesnetsky, *University of Michigan, Ann Arbor*

L. Parent, J. Coffin

Yeast Cell Biology

August 12–17 292 participants

ARRANGED BY **Brenda Andrews,** University of Toronto
Chris Kaiser, Massachusetts Institute of Technology
Mark Winey, University of Colorado, Boulder

This conference was the ninth biannual international meeting devoted to major aspects of cell biology in yeast. It is unusual in that many important areas of cell biology are represented at a single meeting organized around a simple eukaryotic organism, the budding yeast *Saccharomyces cerevisiae*. This year's conference was extraordinary on two fronts. First, the opening session was a celebration of the science of Ira Herskowitz. It began with outstanding retrospective talks by David Botstein and Jeff Strathern on Ira's scientific contributions in the areas of phage genetics and yeast-cell-type determination (these talks are available for viewing on the "The Leading Strand" Web site). Those talks were followed by research presentations from Herskowitz lab alumni who were attending the meeting. The second extraordinary event was the blackout of 2003 that occurred at the end of an afternoon poster session and continued through lunch the following day. The CSHL staff did an outstanding job of on-the-fly reorganization and powering of Grace Auditorium with generators to keep the meeting moving. Not a single presentation was cancelled, and the kitchen hustled to keep everyone fed.

A common interest in one organism, instead of one topic in cell biology, encourages extensive cross-fertilization of ideas, insights, and methodologies, ultimately leading to a more integrated view of eukaryotic cell structure and function. Important insights were further gained by studies in the fission yeast *Schizosaccharomyces pombe* as well as other yeasts. Major areas of interest included the functions of the actin and microtubule cytoskeleton, cell physiology, and the targeting and sorting of proteins in the secretory, endocytotic, and nuclear localization pathways. The coordination of several cellular processes was discussed in the context of the response to the mating pheromone, meiosis, and the cell cycle, with the exit from mitosis being particularly important. Functional genomics, proteomics, and bioinformatics have become a routine part of the yeast cell biologist's repertoire, and genome-wide analysis was featured in many presentations. It is clear that yeast will remain at the forefront of model systems for technology development and mechanistic advances in integrative cell biology for many years. All told, this was once again a very rich, exciting, and memorable meeting with more than 290 scientists in attendance presenting some 235 scientific reports in 101 talks and 134 posters.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations provided core support for this meeting.

B. Andrews, S. Jaspersen, D. Kellogg

PROGRAM

A Celebration of the Science of Ira Herskowitz
Chairperson: D. Botstein, *Princeton University, New Jersey*

Retrospective Talks
Chairpersons: D. Botstein, *Princeton University;* J. Strathern, *National Cancer Institute*

Science Talks: Herskowitz Lab and Alumni

Organelle Biogenesis
Chairperson: L. Weisman, *University of Iowa, Iowa City*

Microtubules and Spindle Pole Bodies
Chairperson: V. Guacci, *Fox Chase Cancer Center, Philadelphia, Pennsylvania*

Cell Cycle Regulation and Dynamics
Chairperson: M. Basrai, *National Cancer Institute, Frederick, Maryland*

Intracellular Trafficking
Chairperson: C. Stirling, *University of Manchester, United Kingdom*

Signaling Cascades, Stress Adaptation, and Cell Polarity
Chairperson: R. Miller, *University of Rochester, New York*

The Kinetochore
Chairperson: B. Goode, *Brandeis University, Waltham, Massachusetts*

Physiology and Cell Regulation
Chairperson: J. Thorner, *University of California, Berkeley*

Chromosomes and Functional Genomics
Chairperson: K. Kaplan, *University of California, Davis*

Actin Cytoskeleton
Chairperson: S. Lemmon, *Case Western Reserve University, Cleveland, Ohio*

C. Stirling, M. Rose

G. Odorizzi, S. van Kreeveld, M. Winey

Poster session

Eukaryotic mRNA Processing

August 20–24 340 participants

ARRANGED BY **Elisa Izaurralde,** EMBL Heidelberg, Germany
Timothy Nilsen, Case Western Reserve University
Donald Rio, University of California, Berkeley

This fourth meeting focused on multiple aspects of mRNA metabolism including the mechanism of splicing, the regulation of alternative splicing, the mechanisms of surveillance, transport, and localization, RNA interference, 3′-end maturation, and decay. In addition, new insights into the coupling of transcription and posttranscriptional processes were presented.

In splicing, proteomic analyses of spliceosomes arrested at different stages of the reaction revealed that the spliceosome is much more dynamic than previously thought; conformational changes are accompanied by the addition and departure of large numbers of proteins. Additionally, new analyses now show that protein-free snRNAs can catalyze branching, strengthening the case that the spliceosome is fundamentally a ribozyme.

Several new experimental approaches including microarrays have been used to analyze alternative splicing. Strikingly, specific introns display different dependencies on splicing factors for efficient excision. Overall, there were few unifying themes for the regulation of splicing; each case seems to be unique and involve unique factors. There was considerable discussion of the relative contribution of positive and negative sequence elements to alternative splicing.

The mechanism of RNA interference continues to be unraveled by both genetics and biochemistry; interesting links between the initiation and effector steps were presented.

In terms of mRNA quality control, several presentations made it quite clear that the cell goes to great lengths to eliminate defective mRNAs, in both the nucleus and cytoplasm. Whether nuclear surveillance involves reading frame recognition is still a matter of hot debate. It is now clear that the exon-junction complex left by the splicing has multiple roles in mRNA quality control both in mediating NMD and in enhancing translation.

E. Izaurralde

The functional links between transcription and posttranscriptional events received much attention. It now seems clear that transcription rate can have profound effects on both the pattern of splicing and the efficiency of the 3′-end formation. A major theme was the integration of all the steps in gene expression.

Other presentations highlighted the impact of the cells environment (e.g., stress) on RNA processing; a factor that inhibits splicing during heat shock was identified, and the differential effect of amino acid starvation of splicing of introns in yeast was revealed by array analysis. Finally, several presentations illustrated the power of informatic approaches in dissecting RNA processing-related problems.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development, branches of the National Institutes of Health; and by the National Science Foundation. The Laboratory would, in addition, like to thank the RNA Society for its support of this meeting. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Capping-3′-end Formation-Polyadenylation
Chairperson: J. Manley, *Columbia University, New York*

Splicing Mechanism
Chairperson: R. Lührmann, *Max-Planck-Institute for Biophysical Chemistry, Göttingen*

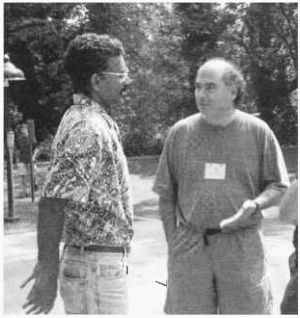
RNA Trafficking and Localization
Chairperson: P. Silver, *Dana Farber Cancer Institute, Boston, Massachusetts*

Regulation of Pre-mRNA Splicing I
Chairperson: J. Valcarcel, *Center for Genomic Regulation, Barcelona, Spain*

mRNA Decay–mRNA Surveillance
Chairperson: D. Tollervey, *University of Edinburgh, United Kingdom*

RNA Interference-regulated Splicing II
Chairperson: M. Moore, *Howard Hughes Medical Institute, Brandeis University, Waltham, Massachusetts*

Global Analysis of RNA Processings: Genomics, Informatics, Evolution
Chairperson: A. Krainer, *Cold Spring Harbor Laboratory*



R. Singh, J. Valcarcel

T. Kashima, F. Kleiman

A. Lingel, L. Unterholzner, G. Tanackovic

Mechanisms of Eukaryotic Transcription

August 27–31 465 participants

ARRANGED BY **Joan Conaway,** Stowers Institute for Medical Research
Richard Treisman, CR-UK London Research Institute
Jerry L. Workman, Stowers Institute for Medical Research

The regulation of gene transcription has a central role in the growth and development of eukaryotic organisms. Transcriptional responses occur as a consequence of cell signaling, environmental stresses, and developmental cues. Thus, the field of transcription encompasses a broad range of study from structural biology to developmental biology. The meeting appropriately covered all aspects of transcription and brought together a diverse group of scientists. It included eight plenary sessions and three poster sessions and started with a session of the most recent detailed structures of RNA polymerase. Two sessions entitled Histone Modification and Chromatin Remodeling covered the role of chromatin in transcription control. The remaining sessions covered the function of upstream transcription activators, transcription complexes, coactivators, elongation, and transcription termination. The final session discussed regulatory networks and mechanisms including genomic and proteomic analyses.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development, branches of the National Institutes of Health; and by the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

R. Singer, J. Bradsher

D. Singer, J. Boss

S. Holmberg, H. Spahr, J. Bève

PROGRAM

Polymerase Structure and Mechanism
Chairperson: K. Yamamoto, *University of California, San Francisco*

Histone Modification
Chairperson: J. Workman, *Pennsylvania State University, University Park*

Activators I
Chairperson: B. Graves, *University of Utah, Salt Lake City*

Transcription Complexes/Mediator
Chairperson: W. Herr, *Cold Spring Harbor Laboratory*

Elongation and Termination
Chairperson: J. Conaway, *Stowers Institute for Medical Research, Kansas City, Missouri*

Chromatin Remodeling
Chairperson: J. Mellor, *University of Oxford, United Kingdom*

Activators II
Chairperson: C. Wolberger, *Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland*

Regulatory Mechanisms and Networks
Chairperson: N. Hernandez, *Howard Hughes Medical Institute, Cold Spring Harbor Laboratory*

M. Collart, G. Prelich

K. Struhl, M. Timmers

Eukaryotic DNA Replication

September 3–7 373 participants

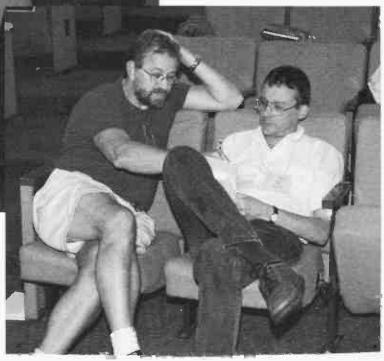
ARRANGED BY **Thomas Kelly,** Memorial Sloan-Kettering Cancer Center
Bruce Stillman, Cold Spring Harbor Laboratory

This was the eighth biannual meeting on eukaryotic DNA replication. Studies of eukaryotic DNA replication are advancing rapidly on many fronts, and this meeting is now established as the most important in the field. A total of 373 investigators participated in the ten scientific sessions and there were nearly 259 platform and poster presentations. Thus, interest in the mechanisms and regulation of DNA replication in eukaryotic cells remains extremely high, and the meeting is playing a key role in fostering the exchange of new ideas and experimental approaches.

Sessions at the meeting included assembling initiation complexes; timing and origin definition; origin and chromosome organization; checkpoint control and stalled forks; polymerases and helicases; fork proteins; and linking replication to other cell division events. Additionally, two poster sessions were held.

Essential funding for the meeting was provided in part by the National Cancer Institute, a branch of the National Institutes of Health; and the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

T. Kelly, J. Yates



G. Freyer, S. Brill

PROGRAM

Assembling Initiation Complexes
Chairperson: J. Campbell, *California Institute of Technology, Pasadena*

Timing and Origin Definition
Chairperson: A. Dutta, *University of Virginia, Charlottesville*

Origin and Chromosome Organization
Chairperson: S. Forsburg, *Salk Institute, La Jolla, California*

Checkpoint Control and Stalled Forks
Chairperson: H. Araki, *National Institute of Genetics, Mishima, Japan*

Polymerases and Helicases
Chairperson: P. Burgers, *Washington University School of Medicine, St. Louis, Missouri*

Fork Proteins
Chairperson: J. Hamlin, *University of Virginia, Charlottesville*

Linking Replication to Other Cell Division Events
Chairperson: J. Rine, *University of California, Berkeley*

Regulation
Chairperson: S. Brill, *Rutgers University, Piscataway, New Jersey*

J. Claycomb, B. Aggarwal

G. Bowman,
V. Bermudez

T. Broker, M. Botchan

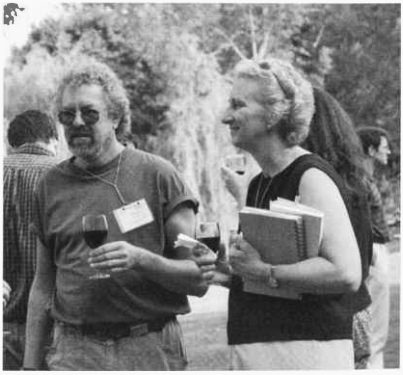
Microbial Pathogenesis and Host Response

September 10–14 265 participants

ARRANGED BY **James Slauch,** University of Illinois
Paula Sundstrom, Ohio State University
Ronald Taylor, Dartmouth Medical School

Throughout recorded history, microbial pathogens have been a major cause of human disease and mortality. However, with the advent of effective antibiotics, it seemed like the war on microbes had been won. Hence, for several decades, health-related research shifted to topics like cancer, heart disease, and genetic diseases. While research in microbial pathogenesis slowed, the microbes continued to evolve. Microbial resistance to antibiotics developed faster than new antibiotics could be made available, and the resistance spread throughout the microbial world. The global expansion of food distribution networks increased the rapid dissemination of microbial pathogens. Simultaneously, emerging microbial pathogens filled new ecological niches, such as indwelling medical devices that provide a surface for biofilms and the growing population of patients who are immunocompromised due to primary infections such as HIV or due to therapies for chronic diseases. Furthermore, recent discoveries have demonstrated that some diseases (e.g., ulcers and coronary heart disease) previously believed to be caused by a genetic predisposition or environmental conditions are actually caused by, or are strongly associated with, microbes. Finally, humans have facilitated the development of microbial pathogens as agents of bioterrorism.

This microbial offensive has summoned a renewed counterattack on microbial pathogens that has intensified during the last several years. A variety of new tools have become available that make it possible to dissect the molecular basis of pathogenesis from both the microbial and host perspectives. This has yielded exciting rapid advances in understanding the basis of pathogenesis for several important infectious diseases. Insight into the molecular mechanisms of pathogenesis has predicted new ways to control infection, including the identification of novel targets for antimicrobials and novel approaches for vaccine development. Nevertheless, many more questions remain unanswered and many pathogens are still poorly understood. Understanding microbial pathogenesis demands a detailed knowledge of the host response as well as the pathogen itself. Both of these perspectives provide potential strategies for solving important clinical problems. Elucidating these distinct aspects of microbial pathogenesis requires an interdisciplinary approach, integrating the fields of microbiology, eukaryotic cell biology, and immunology.



R. Taylor, P. Sundstrom

Unlike the previous meeting, which was rescheduled on short notice because of the September 11, 2001 attack on the World Trade Center, the 2003 meeting proceeded smoothly and was well-attended. Recognition of the importance of program topics by microbial pathogenesis researchers was reflected in the number of participants, which reached the level of the 1999 meeting. The program included Keynote Addresses by Roberto Kolter, Professor of Microbiology and Molecular Genetics at Harvard University, who discussed his work on the interactions that bacteria have with biotic and abiotic surfaces, and A. Casadevall, Albert Einstein College of Medicine, who elaborated on his work with vaccines and immune responses. The importance of the Keynote Address on microbial growth and antibiotic resistance in host niches pointed to the need for new approaches to combat microbial pathogens.

The meeting attracted a diverse group of international scientists who approach the study of bacterial and fungal pathogens from a broad range of perspectives. In the first session, a new scheme that

incorporated the immune status of the host in classifying microorganisms for their ability to induce damage underscored the increasing numbers of compromised hosts and the importance of emerging fungal and bacterial pathogens that prey upon them. Other sessions focused on how gene transfer between distantly related microorganisms contributes to evolution of pathogenic traits; how bacteria build a variety of secretion systems for attaching to and injecting toxic proteins into the host; how bacteria exploit the intracellular environment of host cells; mechanisms of innate host antimicrobial defense and how nonpathogenic, commensal bacteria of the gut induce novel antimicrobial defenses in the host; how yeast can be used to model pathogenic mechanisms of bacteria; how toxins travel within and affect programmed death of host cells; how specific human diseases can be precisely modeled in animals for studying vaccine efficacy and pathogenesis; the molecular structure of protective vaccine antigens; how bacteria and fungi regulate expression of virulence genes; biogenesis of surface structures bacteria and fungi; and how these structures promote survival in the host. The talks and poster sessions generated lively, interactive discussions. Many presentations describing the use of a new method to solve a complex problem led to animated discourse about how the approach could be applied to answer recalcitrant questions about other host-pathogen interactions. Some of these interactions have already produced fruitful scientific collaborations.

Despite the active scientific research on microbial pathogenesis and the impressive progress in this field, it is clear that as one problem is solved, another microbial pathogen will rapidly take its place. Hence, there will be a continual need for the free, interactive exchange of ideas like those that stimulated by this meeting.

This meeting was supported in part by funds from the National Institute of Allergy and Infectious Diseases and the National Institute of Dental and Craniofacial Research, branches of the National Institutes of Health; and the Burroughs Wellcome Fund. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Overview
Chairperson: P. Sundstrom, *Ohio State University, Columbus*

Horizontal Evolution
Chairperson: S. Maloy, *San Diego State University, California*

Mechanisms of Protein Secretion
Chairperson: M. Sandkvist, *American Red Cross, Rockville, Maryland*

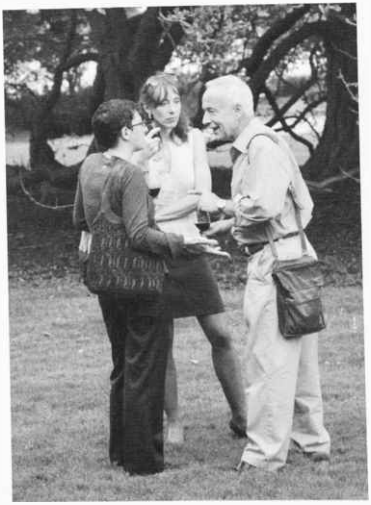
Intracellular Parasites
Chairperson: D. Portnoy, *University of California, Berkeley*

Cellular Microbiology
Chairperson: A. Jerse, *Uniformed Services University, Bethesda, Maryland*

Vaccines/Immune Responses
Keynote Speaker: A. Casadevall, *Albert Einstein College of Medicine, Bronx, New York*

Gene Regulation
Chairperson: L. Kenney, *University of Illinois, Chicago*

Microbial Surfaces
B. Cormack, *Johns Hopkins University School of Medicine, Baltimore, Maryland*



E. Helmerhorst, E. Rappocciolo, F. Oppenheim

Programmed Cell Death

September 17– 21 405 participants

ARRANGED BY **Hermann Steller,** HHMI/Massachusetts Institute of Technology
Craig Thompson, HHMI/University of Chicago
Junying Yuan, Harvard Medical School

This fifth meeting was successful as planned, with only one speaker cancellation due to the threat of Hurricane Isabel, which turned out to be nothing more than a strong wind during the night. The Keynote Speaker in the opening session was 2002 Nobel prize winner H. Robert Horvitz, whose reflection on the explosive growth of the apoptosis field during the last ten years drew much discussion. The opening session was followed by eight oral sessions on the Bcl-2 family; Caspases and other proteases in apoptosis; developmental cell death; signal transduction I; signal transduction II; neuronal cell death; engulfment and alternative pathways of cell death; oncogenes and tumor suppressor genes in apoptosis; and two poster sessions. The invited session leaders are senior investigators of the field. The 47 speakers were selected from 301 abstracts with a good representation of senior lab heads (73%), postdoctoral fellows (13.5%), and graduate students (13.5%). The participants were impressed by the presentation of a significant amount of unpublished information: 30% of the talks consisted of entirely unpublished information and 54% of talks consisted of more than 50% of unpublished information.

This meeting was funded in part by the National Cancer Institute, the National Institute of Aging, and the National Institute of Child Health and Human Development, branches of the National Institutes of Health; Vertex Pharmaceuticals, Ltd.; Idun Pharmaceuticals, Inc.; Cell Signaling Technology, Inc.; and Abgent. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

J. Yuan, H. Steller, C. Thompson

A. Thorburn, Y. Lazebnik

PROGRAM

Keynote Address: Genetic Control of Programmed Cell Death in *C. elegans*
H. Robert Horvitz, *Massachusetts Institute of Technology, Cambridge*

Bcl-2 Family
Chairpersons: S. Korsmeyer, *Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts;* Y. Tsujimoto, *Osaka University Graduate School of Medicine, Japan*

Caspase and Other Proteases in Apoptosis
Chairpersons: Y. Lazebnik, *Cold Spring Harbor Laboratory;* E. Ainemri, *Thomas Jefferson University, Philadelphia, Pennsylvania*

Developmental Cell Death
Chairpersons: M. Hengartner, *University of Zurich, Switzerland;* J. Abrams, *University of Texas Southwestern Medical Center, Dallas*

Signal Transduction I
Chairpersons: X. Wang, *University of Texas Southwestern Medical Center, Dallas;* J. Tschopp, *University of Lausanne, Switzerland*

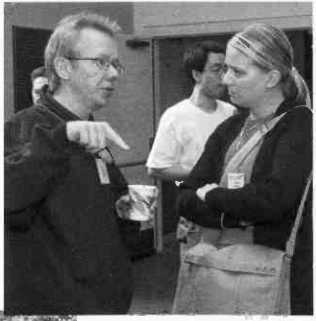
Neuronal Cell Death
Chairpersons: J.M. Hardwick, *Johns Hopkins University, Baltimore, Maryland;* P. Nicotera, *University of Leicester, United Kingdom*

Engulfment and Alternative Pathways of Cell Death
Chairpersons: S. Nagata, *Osaka University Medical School, Japan;* K. White, *Massachusetts General Hospital, Harvard Medical School, Charlestown*

Signal Transduction II
Chairpersons: S. Kornbluth, *Duke University Medical Center, Durham, North Carolina;* D. Green, *La Jolla Institute for Allergy and Immunology, San Diego, California*

Oncogene/Tumor Suppressor Genes in Apoptosis
Chairpersons: S. Lowe, *Cold Spring Harbor Laboratory;* E. White, *Rutgers University, Piscataway, New Jersey*

K. Edwards, D. Sogah



J. Eriksson, A. Meinander

H. Engelberg-Kulka, S. Nagata

FIRST CSHL/WELLCOME TRUST CONFERENCE
Pharmacogenomics

September 24–28 140 participants

ARRANGED BY **David Bentley,** The Sanger Institute, Hinxton
Steve Leeder, Children's Mercy Hospital
Munir Pirmohamed, University of Liverpool
Dick Weinshilboum, Mayo Medical School
Roland Wolf, University of Dundee Biomedical Research Centre

The inaugural meeting on Pharmacogenomics was held at the Wellcome Trust Genome Campus in Hinxton, U.K. and was a joint project of CSHL and the Wellcome Trust. The meeting was arranged to have a strong multidisciplinary focus and to address diverse issues related to applying genomics to variable drug response in the human population. Key subject areas of the meeting included genes and sequence variants, genotype and phenotype correlations, clinical association studies, molecular mechanisms of drug action and response, adverse drug reactions, clinical applications, drug development and use (including regulatory issues), commercial/economic/ELSI implications, educational/software/informatics, and disease entities. A prominent emphasis of the meeting was to explore the impact of genomics on the field of pharmacogenetics and to focus on the benefits and challenges that are currently most closely relevant to the general public and to the medical field, and to see how these relate to the goals of pharmaceutical and other commercial entities.

The meeting was subscribed to by 140 attendees and included participants from 15 different countries representing Australia, North America, Central America, Asia, Europe, and Scandinavia, including 20% representation of students and postdocs. Representation of women at the first meeting was an encouraging 38% of attendees. The meeting was warmly received by all the participants, with the hope that there is further opportunity for many younger members of their laboratories and collaborating groups to attend in the future. Many of these researchers are renowned in their own field but had little exposure to other disciplines before this meeting.

PROGRAM

Welcome
Chairperson: David Bentley, *Wellcome Trust Sanger Institute*

Introduction
Chairpersons: A. Breckenridge, *University of Liverpool, United Kingdom;* C.R. Wolf, *University of Dundee, United Kingdom*

Genes and Sequence Variants
Chairpersons: K. Giacomini, *University of California, San Francisco;* J. Licinio, *David Geffen School of Medicine, University of California, Los Angeles*

Drug Response
Chairpersons: A. Somogyi, *University of Adelaide, Australia;* G. Smith, *University of Dundee, United Kingdom*

Adverse Drug Reactions
Chairpersons: M. Eichelbaum, *Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany;* D. Kaufman, *Boston University, Massachusetts*

Genotype to Phenotype
Chairpersons: S. Liggett, *University of Cincinnati, Ohio;* A. Rane, *Huddinge University Hospital, Stockholm, Sweden*

Diseases and Future Challenges
Chairpersons: B. Ponder, University of Cambridge, United Kingdom; S. Weiss, *Brigham and Women's Hospital, Boston, Massachusetts*

Hinxton, U.K.

Neurobiology of *Drosophila*

October 1–5 407 participants

ARRANGED BY **Thomas Schwarz,** Children's Hospital Harvard
Jessica Treisman, New York University School of Medicine

The goal of this 2003 meeting, as it has been since its inception, was to foster communication of ideas, techniques, and new discoveries within the field of *Drosophila* neurobiology. The meeting was structured with platform and poster presentations by a variety of researchers including graduate students, postdoctoral fellows, and junior and senior faculty. All platform presentations were selected from the abstracts submitted by the session chairs. The topics for the platform sessions were chosen from the areas where exciting advances are being made in understanding molecular and cellular mechanisms: neurophysiology, behavior, sensory systems, axon guidance, synapse formation and function, neuronal and glial determination, and neuronal cell biology and pathology. The research reported relied on a wide range of techniques, including genetic, molecular, cellular, neurophysiological, behavioral, and genomic approaches to basic questions of nervous system development and function. Among the highlights of the meeting were presentations of new and exciting developments, including visualization of synaptic transmission at the level of individual boutons; identification of new molecules contributing to axon guidance; and genomic analysis of glial cell functions. The Elkins Plenary Lecture, which was presented by recent Ph.D. graduate Greg Jefferis, described exciting work on the establishment of an olfactory map in the brain before the arrival of olfactory neuron axons. The environment of the meeting allowed many opportunities for informal discussions among all participants. The high quality of the presentations, novel findings, and new techniques discussed at the meeting demonstrated the vitality of *Drosophila* research. The character of the discussions led to the cross-fostering of ideas that was valuable to everyone in the field.

J. Price, A. Sehgal

J. Treisman, T. Schwarz

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health; and the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Behavior
Chairperson: A. Sehgal, *University of Pennsylvania, Philadelphia*

Axon Guidance
Chairperson: D. Van Vactor, *Harvard Medical School, Boston, Massachusets*

Elkins Memorial Lecture
G. Jefferis, *Stanford University, California*

Neuronal Function
Chairperson: L. Griffith, *Brandeis University, Waltham, Massachusetts*

Formation and Function
Chairperson: A. DiAntonio, *Washington University School of Medicine, St. Louis, Missouri*

Sensory Systems
Chairperson: L. Vosshall, *The Rockefeller University, New York*

Neuronal Cell Biology and Pathology
Chairperson: M. Bhat, *University of North Carolina, Chapel Hill*

Neuronal and Glial Determination
Chairperson: A. Jarman, *University of Edinburgh, United Kingdom*

D. Eberl, J. Simpson

I. Meinertzhagen, T. Clandinin

Caricaturist in action!

Molecular Approaches to Vaccine Design

December 4–7 99 participants

ARRANGED BY **Peter Beverley,** Edward Jenner Institute for Vaccine Research
Dennis Burton, Scripps Research Institute
Emilio Emini, Merck Research Laboratories

This third winter conference was held in a snow blizzard that turned the Laboratory into a setting from a Christmas card. The emphasis of the meeting was on how to exploit the full new developments in molecular and structural biology, to enable the design of new generations of vaccines that will be safe and effective in humans. The meeting included sessions on HIV and emerging pathogens, B cells and vaccines, T cells and vaccines, new vaccines in late development, the future of vaccines, and new developments in infectious disease and vaccinology. Much new information on ingenious replicating or nonreplicating vectors for antigen expression was presented, as was encouraging data on understanding of T- and B-cell responses to model antigens and vaccines. Nevertheless, it emerged that preclinical models do not always predict whether an immunization strategy will generate a strong immune response in humans. An important step in vaccine development therefore remains the intensive study of vaccinated humans in early-phase small-scale trials. A challenging talk given by a vaccine policy expert outlined the financial hurdles inherent in vaccine development and the need for imaginative partnerships among industry, governments, international bodies, and charities to develop vaccines needed in poorer countries.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations provided core support for this meeting.

K. Smith, P. Beverley

PROGRAM

HIV and Emerging Pathogens
Chairperson: G. Nabel, *NIAID, National Institutes of Health, Bethesda, Maryland*

B Cells and Vaccines
Chairperson: D. Burton, *Scripps Research Institute, La Jolla, California*

T Cells and Vaccines
Chairperson: M. Bevan, *Howard Hughes Medical Institute, University of Washington, Seattle*

New Vaccines in Late Development
Chairperson: P. Beverley, *Edward Jenner Institute for Vaccine Research, Compton, United Kingdom*

Future of Vaccines
Chairperson: P. Beverley, *Edward Jenner Institute for Vaccine Research, Compton, United Kingdom*

New Developments in Infectious Disease and Vaccinology
Chairperson: E. Emini, *Merck Research Laboratories, West Point, Pennsylvania*

J. Almond, N. Almond

Rat Genomics and Models

December 11–14 164 participants

ARRANGED BY **Howard Jacob,** Medical College of Wisconsin
Douglas Vollrath, Stanford University School of Medicine

This third winter biotechnology conference followed on from the successful inaugural meetings in December 1999 and 2001. This conference series is the first to focus exclusively on the rat as a model organism for biological research and seeks to bring together the vast body of research that continues to be generated in the area of rat physiology, pathophysiology, toxicology, neuroscience, etc., with new genetic resources and genomic tools now being developed for the rat. Topics at the meeting included Pharmacogenomics and Risk Assessment; Neuroscience; Toward ES cells and Knockouts; Comparative Mapping; Models and Analysis; Rat Cloning; Gene Transfer and Transgenesis; and Rat Genome Sequencing. The meeting also included a special workshop on resources for the community.

This meeting was funded in part from the National Center for Research Resources, a branch of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

U. Vitt, D. Vollrath

V. Kren, F. Stahl

PROGRAM

Welcome
Chairpersons: H. Jacob, *Medical College of Wisconsin, Milwaukee;* D. Vollrath, *Stanford University School of Medicine, California*

Sequencing the Rat
Chairperson: S. Old, *NHLBI, National Institutes of Health, Bethesda, Maryland*

Comparative Genomics
Chairperson: D. Vollrath, *Stanford University School of Medicine, California*

Models: Neuroscience
Chairperson: M.-T. Perez, *Lund University, Sweden*

Models: Kidney/Cancer
Chairpersons: P. Harris, *Mayo Clinic, Rochester, Minnesota;* G. Levan, *Göteborg University, Sweden*

Resources
Chairperson: L. Riley, *University of Missouri, Columbia*

Models: Diabetes/Lipids/Cardiovascular
Chairperson: A. Kwitek, *Medical College of Wisconsin, Milwaukee*

Augmenting and Inhibiting Gene Function
Chairpersons: B. Davidson, *University of Iowa College of Medicine, Iowa City;* M. Gould, *University of Wisconsin, Madison*

Arthritis/Immunity
Chairperson: M. Griffiths, *University of Utah, Salt Lake City*

Expression Profiling and Pharmacogenomics
Chairperson: C. Kendziorski, *University of Wisconsin, Madison*

C. Moreno-Quinn, M. Tschannen, J. Lazar

POSTGRADUATE COURSES

The Postgraduate Courses program at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that universities do not adequately treat them. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together course faculty from many laboratories around the world and supplement this staff with a series of seminar speakers.

Acquiring and Analyzing Genomic Sequence Data

March 11–27

INSTRUCTORS
E. Mardis, Washington University, St. Louis, Missouri
W.R. McCombie, Cold Spring Harbor Laboratory
J. McPherson, Washington University, St. Louis, Missouri

ASSISTANTS
V. Balija, Cold Spring Harbor Laboratory
L. Courtney, Washington University School of Medicine, St. Louis, Missouri
W. Courtney, Washington University School of Medicine, St. Louis, Missouri
M. De la Bastide, Cold Spring Harbor Laboratory
L. Nascimento, Cold Spring Harbor Laboratory
A. O'Shaughnessy, Cold Spring Harbor Laboratory
L. Palmer, Cold Spring Harbor Laboratory
L. Spiegel, Cold Spring Harbor Laboratory
T. Zutavern, Cold Spring Harbor Laboratory

This year's course consisted of four modules, which featured (1) production sequencing and sequence finishing, (2) sequence variation analysis, (3) cross-species sequence comparison, and (4) gene prediction and annotation. The first part of the course emphasized the technical and management aspects of large-scale physical mapping and sequencing projects including polymorphism detection. In the second part, computational analysis of sequence data was the focus, including sequence variation (such as single-nucleotide polymorphisms [SNPs]), comparative genomics, gene prediction, and sequence annotation. Where appropriate, the computational analysis was coupled with data acquisition. A sequencing project was carried out during the course, with emphasis on the technical nuances of large-scale sequencing. In past years, this phase has targeted a bacterial artificial chromosome clone from various species, including *Arabidopsis thaliana*, mouse, chimpanzee, and human. Techniques for generating both "working draft" and completely finished genome sequences were covered. Whole-genome shotgun and clone-based sequencing methods utilizing capillary-based sequence acquisition was taught.

Sequence variation analysis featured SNP generation and detection methods. A laboratory project included sequencing of targeted regions from multiple individuals and additional SNP analysis. Computational analysis focused on using readily available tools for comparative genomics, gene prediction, and annotation methods. Data from the course production sequencing module were utilized, as well as examples from the publicly accessible sequence archives. Sequence homology was used to analyze orthologous and parologous sequences. Ab initio gene prediction, as well as supporting evidence using expressed sequence tag (EST), mRNA, and homologous sequences, was profiled. These modules focused primarily on the use of the tools themselves and only generally on the underlying algorithms.

PARTICIPANTS

Adeniji-Adele, A., B.S., M.S., Lancaster Laboratories, Richmond, Virginia
Crayton, M., III, B.S., University of North Carolina, Chapel Hill
Geng, T., B.A., M.S., Comparative Genomics Lab., Blacksburg, Virginia
Gore, M., B.S., M.S., Pioneer, Johnston, Iowa
Jiang, C., B.S., University of Virginia, Charlottesville
Larsen, C., B.S., National Institute for Medical Research, London, United Kingdom
Long, E., B.A., Virginia Tech, Blacksburg
Mavrodi, D., B.S., Ph.D., Washington State University, Pullman
Mecklenburg Serkovic, E., B.A., Universidad Peruana Cayetano Heredia, Lima, Peru
Raudsepp, T., B.S., Ph.D., Texas A&M University, College Station
Wang, Q.J., B.M., Ph.D., Lawrence Berkeley National Laboratory, Berkeley, California

SEMINARS

Beier, D., Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts: Mouse genomics.
Hannon, G., Cold Spring Harbor Laboratory: RNAi: Mechanism and application.
Hirschhorn, J., Harvard University, Boston, Massachusetts: SNPs, patterns of genetic variation, and complex traits.
Martienssen, R., Cold Spring Harbor Laboratory: Chromatin silencing.
Stein, L., Cold Spring Harbor Laboratory: The generic model organism database suite of bioinformatics tools.
Vidal, M., Dana-Farber Cancer Institute/Harvard Medical School, Boston, Massachusetts: From genomes to systems biology.
Watson, J., Cold Spring Harbor Laboratory: The Human Genome Project.

Protein Purification and Characterization

April 2–15

INSTRUCTORS

R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S. Lin, M.D. Anderson Cancer Center/University of Texas, Houston
K. Severinov, Waksman institute, Rutgers University, Piscataway, New Jersey

ASSISTANTS

V. Bergendahl, University of Wisconsin, Madison
M. Galfione, M.D. Anderson Cancer Center/University of Texas, Houston
N. Thompson, University of Wisconsin, Madison
G. Ratnaparkhi, University of California, Los Angeles
W. Tsai, M.D. Anderson Cancer Center/University of Texas, Houston
C. Winkler, University of California, Los Angeles
N. Zenkin, Waksman institute, Rutgers University, Piscataway, New Jersey

This course was intended for scientists who are not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations, including (1) a regulatory protein from muscle tissue, (2) a sequence-specific, DNA-binding protein, (3) a recombinant protein overexpressed

in *E. coli*, and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques included precipitation by salts, pH, and ionic polymers; ion-exchange, gel filtration, hydrophobic interaction, and reverse-phase chromatography; lectin affinity, ligand affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis and electroblotting; and high-performance liquid chromatography. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were utilized to include immunological and biochemical assays, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology.

PARTICIPANTS

Apionishev, S., B.S., Ph.D., Columbia University, New York
Belanger, M., B.S., Ph.D., University of Florida, Gainesville
Dale, B., B.A., M.S., Columbia University, New York
Grace, W., B.S., USAMRIID, Detrick, Maryland
Guo, H., M.D., Ph.D., Duke University, Durham, North Carolina
Karger, A., Ph.D., Applied Biosystems, Foster City, California
Lou, J., B.S., Washington University School of Medicine, St. Louis, Missouri
Miller, D., B.S., Ph.D., National Institute on Aging, Bethesda, Maryland
Needleman, D., B.A., University of California, Santa Barbara
Petersen, J., B.S., Ph.D., Peterson Institute for Cancer Research, Manchester, United Kingdom
Sattlegger, E., Ph.D., NICHD, National Institutes of Health, Bethesda, Maryland
Tsang, P., B.A., Ph.D., University of Cincinnati, Ohio
Vullhorst, D., Ph.D., NICHD, National Institutes of Health, Bethesda, Maryland
Weininger, S., B.S., Ph.D., The Gene Pool, Inc., Seattle, Washington
Young, I., Ph.D., University of Liverpool, United Kingdom
Zarivach, R., B.S., Ph.D., Weizmann Institute of Science, Rehovot, Israel

SEMINARS

Burgess, R., University of Wisconsin: Overview of protein purification and immunoaffinity purification. Biochemical studies of RNA polymerase/σ factor interactions.
Courey, A., University of California, Los Angeles: Wrestling with SUMO function.
Guidotti, G., Harvard University, Cambridge, Massachusetts: Membrane proteins and extracellular ATP.
Hart, G., Johns Hopkins School of Medicine, Baltimore, Maryland: Dynamic interplay between O-glycosylation and O-phosphorylation in cell signaling and metabolic regulation.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Proteins in 3-D.
Lin, S., M.D. Anderson Cancer Center, Houston, Texas: Prostate cancer bone metastasis.
Mische, S., Boehringer-Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut: Proteomics and why pharmaceutical companies are interested in it.
Severinov, K., Waksman Institute, Rutgers, Piscataway, New Jersey: Structure-function analysis of transcriptional machinery in bacteria.
Stillman, B., Cold Spring Harbor Laboratory: Understanding chromosome duplication in human cells: Combining biochemistry, genetics, and cell biology.

Cell and Developmental Biology of *Xenopus*

April 5–15

INSTRUCTORS **K. Cho,** University of California, Irvine
J. Christian, Oregon Health Sciences University, Portland

ASSISTANTS **I. Blitz,** University of California, Irvine
G. Dalgin, Oregon Health and Sciences University, Portland
C. Degnin, Oregon Health and Sciences University, Portland
T. Nakayama, University of Virginia, Charlottesville

The frog *Xenopus* is an important vertebrate model for studies of maternal factors, regulation and molecular mechanisms of tissue inductions and regulation of cell-fate decisions. In addition, *Xenopus* oocytes and embryos provide a powerful system in which to conduct a number of cell biological and gene regulation assays. This course provided extensive laboratory exposure to the biology, manipulation, and use of oocytes and embryos of *Xenopus*. The course consisted of intensive laboratory sessions, supplemented by daily lectures and demonstrations from experts in cellular, experimental, and molecular development. Areas covered included (1) care of adults; (2) oocyte isolation and embryo production; (3) stages of embryonic development and anatomy; (4) whole-mount in situ hybridization and immunocytochemistry; (5) microinjection of eggs and oocytes with lineage tracers, DNA constructs, mRNA, and antisense oligonucleotides; (6) micromanipulation of embryos, including explant and transplantation assays; (7) in vivo time-lapse confocal imaging; (8) preparation of transgenic embryos; and

(9) use of *Xenopus tropicalis* for genetic analyses. This course was suited both for investigators who have had no experience with *Xenopus* and for those who have worked with *Xenopus* and wished to learn new and cutting-edge techniques. All applicants had current training in molecular biology and some knowledge of developmental biology.

PARTICIPANTS

Collart, C., Ph.D., Wellcome Trust, Cambridge, United Kingdom
Gauley, J., B.S., University of Waterloo, Ontario, Canada
Gorgoni, B., B.S., Ph.D., Medical Research Council, Edinburgh, United Kingdom
Kennedy, E., B.A., Ph.D., University College Dublin, Belfield, Ireland
Klisch, T., B.S., University of Goettingen, Germany
Koyabu, Y., B.S., Ph.D., RIKEN Brain Science Institute, Japan
Kuan, C., M.D., Ph.D., Cincinnati Children's Hospital Research Foundation, Ohio
Lau, J.M.C., B.A., Ph.D., Washington University, St. Louis, Missouri
Martinez, S., B.S., Ph.D., Boston College, Chestnut Hill, Massachusetts
Metcalfe, M.J., M.S., Ph.D., Catholic University of Chile, Santiago
Pradda, C., M.D., SUNY at Buffalo, New York
Rodrigues, C., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Reggiani, L., M.S., Swiss Federal Institute of Technology, Zurich, Switzerland
Shiotsugu, J., B.S., Ph.D., University of California, Irvine
Stebbins-Boaz, B., B.A., Ph.D., Willamette University, Salem, Oregon
Woltering, J., M.S., Hubrecht Laboratory, Utrecht, The Netherlands

SEMINARS

Cho, K., University of California, Irvine: Phylogenetic footprinting and DNA microarray analysis to study *Xenopus* development.
Christian, J., Oregon Health Sciences University, Portland: Introduction to early embryology and dorsoventral patterning. Tissue-specific use of an upstream cleavage site within the prodomain regulates BMP-4 activity and signaling range.
Keller, R., University of Virginia, Charlottesville: Early morphogenesis of *Xenopus*.
King, M.L., University of Miami School of Medicine, Florida: To be or not to be totipotent: Formation of the *Xenopus* germ line.
Kroll, K., Washington University School of Medicine, St. Louis, Missouri: Subdividing the ectoderm: Connecting signals and responses during neural plate formation.
Whitman, M., Harvard Medical School, Boston, Massachusetts: Studying nodal signaling in the early embryo.
Zorn, A., Children's Hospital Medical Center of Cincinnati, Ohio: Sox17 in endoderm formation.

Structure, Function, and Development of the Visual System

June 4–17

INSTRUCTORS **A.K. McAllister,** University of California, Davis
W.M. Usrey, University of California, Davis

ASSISTANT **H. Alitto,** University of California, Davis

This lecture/discussion course explored the functional organization and development of the visual system as revealed by the use of a variety of anatomical, physiological, and behavioral methods. It was designed for graduate students and more-advanced researchers who wished to gain a basic understanding of the biological basis for vision and to share in the excitement of the latest developments in this field. Topics included phototransduction and neural processing in the retina; functional architecture of striate cortex; cellular basis of cortical receptive field properties; the anatomy, physiology, and perceptual significance of parallel pathways; functional parcellation of extrastriate cortex; the role of patterned neuronal activity in the development of central visual pathways; and molecular mechanisms of development and plasticity in the visual system. The course was held at the Laboratory's Banbury Conference Center.

PARTICIPANTS

Akula, J., B.A., Northeastern University, Boston, Massachusetts
Blanche, T., B.S., University of British Columbia, Vancouver, Canada
Chong, S., M.A., Princeton University, New Jersey
Grubb, M., M.S., University of Oxford, United Kingdom
Hansen, C., B.A., University of California, Davis
Heimel, J., Ph.D., Brandeis University, Waltham, Massachusetts
Hofer, S., Ph.D., Max-Planck Institute of Neurobiology, Martinsried, Germany
Kee, C., M.A., University of Houston, Texas
Kim, M., M.S., University of Minnesota
Krahe, T., Ph.D., Virginia Commonweath University, Richmond
Landi, S., B.S., Scuola Normale Superiore, Pisa, Italy
Lesica, N., B.S., Harvard University, Cambridge, Massachusetts
Moore, B., B.A., University of California, Davis
Mueller, I., B.A., Otto-von-Guericke University of Magdeburg, Germany
Natale, L., M.A., University of Genoa, Italy
Nicol, D., B.S., Glasgow Caledonian University, Scotland
Rathbun, D., B.S., University of California, Davis
Sohn, W., M.A., Rutgers University, Piscataway, New Jersey
Zarrinpar, A., A.B., The Salk Institute for Biological Studies, La Jolla, California

SEMINARS

Bear, M., Brown University, Providence, Rhode Island: Cellular mechanisms underlying visual cortical plasticity.
Britten, K., University of California, Davis: Neural mechanisms for processing of optic flow.
Carandini, M., New York University, New York: A synaptic explanation of suppression in visual cortex.
Chapman, B., University of California, Davis: How the visual system got its stripes.
Constantine-Paton, M., Massachusetts Institute of Technology, Cambridge: Cellular mechanisms of visual system development.
Dacey, D., University of Washington, Seattle: Parallel pathways for spectral coding in primate.
Dan, Y., University of California, Berkeley: Spike-timing-dependent synaptic plasticity in visual cortex.
Fitzpatrick, D., Duke University, Durham, North Carolina: Functional maps and patterns of connectivity in primary visual cortex.
Hirsch, J., University of Southern California, Los Angeles: Synaptic physiology and receptive field structure in the early visual pathway of the cat.
Horton, J., University of California, San Francisco: Development of ocular dominance columns in visual cortex.
Masland, R., Harvard University, Boston, Massachusetts: Neuronal diversity in the retina.
Mason, C., Columbia University, New York: Retinal development and molecular mechanisms governing crossing at the optic chiasm.
Maunsell, J., Baylor College of Medicine, Houston, Texas: Attention and visual responses in extrastriate.
McAllister, K., University of California, Davis: The role of neurotrophins in activity-dependent competition in the visual cortex.
Miller, K., University of California, San Francisco: Neural mechanisms of orientation selectivity in the visual cortex.
Movshon, J.A., New York University, New York: Behavioral choice and the visual responses of neurons in macaque MT.
Neitz, J., Medical College of Wisconsin, Milwaukee: Evolution of trichromacy in vertebrates.
Nelson, S., Brandeis University, Waltham, Massachusetts: Mechanisms of syaptic plasticity in the cerebral cortex.
Ringach, D., University of California, Los Angeles: Response properties of macaque primary visual cortex.
Shadlen, M., University of Washington, Seattle: Neural mechanisms that underlie decisions about sensory stimuli.
Sherman, S.M., Stony Brook University, New York: The functional organization of thalamocortical relays.
Stone, L., NASA Ames Research Center, Moffett Field, California: Eye movements, visual search, and motion.
Usrey, M., University of California, Davis: Functional properties of neural circuits in the visual system.
Victor, J., Cornell University Medical College, New York: How the brain uses time to represent and process visual information.
Williams, D., University of Rochester, New York: Functional properties of the photoreceptor.

Advanced Bacterial Genetics

June 4–24

INSTRUCTORS **K. Hughes,** University of Washington, Seattle
U. Jenal, University of Basel, Switzerland
K. Pogliano, University of California, San Diego

ASSISTANTS **T. Fuchs,** University of Basel, Switzerland
A. Rubio, University of California, San Diego
E. Smith, University of Washington, Seattle

The course presented logic and methods used in the genetic dissection of complex biological processes in eubacteria. Laboratory methods used included classical mutagenesis using transposons, mutator strains, and chemical and physical mutagens; the mapping of mutations using genetic and physical techniques; modern approaches to the generation and analysis of targeted gene disruptions and fusions using polymerase chain reaction (PCR) and cloning methods; epitope insertion mutagenesis; and, site-directed mutagenesis. Key components of the course were the use of sophisticated genetic methods in the analysis of model eubacteria and the use of the wealth of new genomic sequence information to motivate these methods. Invited lecturers presented various genetic approaches to study eubacterial mechanisms of metabolism, development, and pathogenesis.

PARTICIPANTS

Altschuler, S., Ph.D., Harvard University, Cambridge, Massachusetts
Baltrus, D., B.A., University of Oregon, Eugene
Bouchet, V., B.S., Boston University, Massachusetts
Denef, V., M.S., University of Ghent, Belgium/Michigan State University, E. Lansing
Gitai, Z., B.S., Stanford University, California
Hekstra, D., M.S., The Rockefeller University, New York
Johnson, A., B.S., Duke University, Durham, North Carolina
Lloyd, L., B.S., Imperial College London, United Kingdom
Marcusson, L., M.S., Uppsala University, Sweden
Persson, O., M.S., Gothenburg University, Sweden
Purdy, A., Ph.D., Scripps Institution of Oceanography, La Jolla, California
Rajagopal, S., B.S., University of Nebraska, Lincoln
Sello, J., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Vlamis, A., Ph.D., Karolinska Institutet, Stockholm, Sweden
Wilson, M., Ph.D., The Colorado College, Colorado Springs
Yook, K., Ph.D., University of Oxford, United Kingdom

SEMINARS

Bassler, B., Princeton University, New Jersey: Tiny conspiracies: Cell-to-cell communication in bacteria.
Gottesman, S., National Cancer Institute, Bethesda, Maryland: Genetic circuits with small RNA switches.
Hengge-Aronis, R., Free University of Berlin, Germany: Cellular information processing in regulatory networks: The general stress response in *E. coli*.
Hughes, K., University of Washington, Seattle: Gene regulation coupled to the development of the flagellar organelle in *Salmonella*.
Jenal, U., University of Basel, Switzerland: Polar differentiation in *Caulobacter crescentus*.
Kolter, R., Harvard Medical School, Boston, Massachusetts: A rose is a rose is a rose...but is a biofilm a biofilm?
Manoil, C., University of Washington, Seattle: Genomic genetics of *Pseudomonas aeruginosa*.
Metcalf, W., University of Illinois, Urbana: Genetic analysis of methanogenesis in *Methanosarcina* species.
Olivera, B., University of Utah, Salt Lake City: The marine Cone snails use a combinatorial library mechanism to generate a plethora of deadly neuropeptides.
Parkinson, J., University of Utah, Salt Lake City: Bacterial chemotaxis: Collaborative signaling by chemoreceptor teams.
Pogliano, K., University of California, San Diego: Dynamic rearrangement of the bacterial cell during *B. subtilis* sporulation.
Slauch, J., University of Illinois, Urbana: Role of Gifsy-2 bacteriophage in *Salmonella* virulence.
Youderian, P., Texas A&M University, College Station: Efflux pumps and antibiotic resistance in *Salmonella*.

Molecular Embryology of the Mouse

June 4–24

INSTRUCTORS	**T. Lufkin,** Mt. Sinai School of Medicine, New York **C. Stewart,** National Cancer Institute, Frederick, Maryland
CO-INSTRUCTORS	**B. Capel,** Duke University Medical Center, Durham, North Carolina **M. Shen,** UMDNJ–Robert Wood Johnson Medical School, Piscataway
ASSISTANTS	**D. Escalante-Alcalde,** Instituto De Fisiologia Celular-UNAM, Mexico **R. Robledo,** Mt. Sinai School of Medicine, New York **L. Vong,** Albany Medical College, New York

This intensive laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse embryonic development. Laboratory components provided an introduction into the technical aspects of working with and analyzing mouse embryos, and lectures provided the conceptual basis for contemporary research in mouse development. Experimental techniques were covered in the practicals including in vitro culture and manipulation of preimplantation and postimplantation embryos, transgenesis by DNA microinjection, embryo transfer, establishment, culture and genetic manipulation of embryonic stem cells, production of chimeras by aggregation with and injection of embryonic stem cells and the analysis of development by whole-mount in situ hybridization, skeletal preparation, and transgene expression. This year's speakers were Richard Behringer, Timothy Bestor, James Cross, Charles Emerson, Thomas Gridley, Brigid Hogan, Monica Justice, Robb Krumlauf, Mark Lewandoski, Robin Lovell-Badge, Anne McLaren, Andras Nagy, Nadia Rosenthal, John Schimenti, Heidi Scrable, Austin Smith, Davor Solter, A. Francis Stewart, Patrick Tam, David Threadgill, and Luk Van Parijs.

PARTICIPANTS

Agostino, A., B.S., Neurologica Institute, Milan, Italy
Beck, C., Diploma Central Institute for Mental Health, Mannheim, Germany
Carmell, M., B.A., Cold Spring Harbor Laboratory
Cheloufi, S., B.S., Cold Spring Harbor Laboratory
Ciaudo, C., M.S., L'Institut Pasteur, Paris, France
Jones, E., M.S., California Institute of Technology, Pasadena
Juhl, K., M.S., University of Colorado Health Sciences Center, Denver
Lanner, F., B.S., Karolinska Institutet, Stockholm, Sweden
McBratney, B., B.A., Harvard University, Cambridge, Massachusetts
Morris, L., M.S., University of Edinburgh, United Kingdom
Pasqualetti, M., Ph.D., University of Pisa, Italy
Ryan, K., Ph.D., CHOP and University of Pennsylvania, Philadelphia
Shureiqi, I., M.D., University of Texas/MD Anderson Cancer Center, Houston
Woodhoo, A., B.S., University College London, United Kingdom

SEMINARS

Behringer, R., The University of Texas, Houston: Transgenesis and gene targeting.
Bestor, T., Columbia University, New York: Epigenetics.
Capel, B., Duke University Medical Center, Durham, North Carolina: Gonad organogenesis.
Cross, J., University of Calgary, Canada: Development of extraembryonic tissue.
Emerson, C., University of Pennsylvania, Philadelphia: Muscle development.
Gridley, T., The Jackson Laboratory, Bar Harbor, Maine: Notch signaling.
Hogan, B., Duke University Medical Center, Durham, North Carolina: Genetic regulation of organogenesis.
Justice, M., Baylor College of Medicine, Houston, Texas: ENU mutagenesis.
Krumlauf, R., Stowers Institute for Medical Research, Kansas City, Missouri: Patterning in the CNS and vertebrate embryo.
Lewandowski, M., National Cancer Institute, Frederick, Maryland: Molecular genetics of limb development.
Lovell-Badge, R., MRC National Institute for Medical Research, United Kingdom: Sex determination: Building genetic pathways.
Lufkin, T., Mount Sinai School of Medicine, New York: Homeobox genes and patterning.
Magnuson, T., University of North Carolina, Chapel Hill: Model system mouse—A synthesis and example.
McLaren, A., Wellcome/CRC Institute, Cambridge, United Kingdom: Germ cells and imprinting.
Nagy, A., Samuel Lunenfeld Research Institute, Toronto, Canada: Embryonic stem cells and the biology of chimeras.
Rosenthal, N., European Molecular Biology Laboratory, Italy: Heart development.
Schimenti, J., The Jackson Laboratory, Bar Harbor, Maine: Modern and classical forward approaches in mice for identifying novel developmental genes.
Scrable, H., University of Virginia, Charlottesville: Emerging technologies in transgenic mice.
Shen, M., UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey: Left-right patterning.
Smith, A., University of Edinburgh, United Kingdom: Stem cells.
Solter, D., Max-Planck-Institüt fur Immunbiologie, Freiburg, Germany: Cloning and preimplantation development.
Stewart, C., National Cancer Institute, Frederick, Maryland: Implantation.
Tam, P., University of Sydney, Australia: Postimplantation development and body plan. Mouse models and experimental manipulation.
Threadgill, D., University of North Carolina, Chapel Hill: Bioinformatics.
Van Parijs, L., Massachusetts Institute of Technology, Cambridge: Lentivirus-based transgenesis.

Ion Channel Physiology

June 4–24

INSTRUCTORS **M. Farrant,** University College London, United Kingdom
M. Hausser, University College London, United Kingdom
N. Spruston, Northwestern University, Evanston, Illinois

ASSISTANTS **B. Clark,** University College London, United Kingdom
T. Jarsky, Northwestern University, Evanston, Illinois
B. Stell, University of California, Los Angeles

The primary goal of this course was to investigate, through lectures and laboratory work, the properties of ion channels that allow neurons to carry out their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitter at central and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, (3) respond to neuromodulators with changes in functional properties, or (4) are developmentally required and regulated. The research interests of guest lecturers reflected these areas of emphasis.

The laboratory component of the course introduced students to electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion-channel activity in acutely isolated or cultured cells or neurons in brain slice preparations. Different recording configurations were used (e.g., whole-cell, cell-free, and nucleated patches)

to examine macroscopic or single-channel activity. Similarly, various methods of ligand and drug application were demonstrated. The advantages and disadvantages of each method, preparation, and recording technique were considered in relation to the specific scientific questions being asked.

PARTICIPANTS

Biro, A., M.D., Institute of Experimental Medicine, Budapest, Hungary
Ellis-Davies, G., Ph.D., Drexel University College of Medicine, Philadelphia, Pennsylvania
Gray, N., B.S., Mayo Graduate School, Rochester, Minnesota
Jiang, J., B.S., Columbia University, New York
Kaczorowski, C., B.A., Northwestern University, Chicago, Illinois
Keck, T., B.A., Boston University, Massachusetts
Mandolesi, G., B.S., Scuola Normale Superiore, Pisa, Italy
Margolis, D., B.S., University of Washington, Seattle
Marinelli, M., Ph.D., FUHS/The Chicago Medical School, Illinois
Moenter, S., Ph.D., University of Virginia, Charlottesville
Phillips, M., B.S., Massachusetts Institute of Technology, Cambridge
Si, K., Ph.D., Columbia University, New York

SEMINARS

Borst, G., Universiteit van Amsterdam, The Netherlands: Presynaptic calcium channels and transmitter release.
Jonas, P., Albert-Ludwigs-Universitat, Freiburg, Germany: Glutamate receptors and synaptic transmission.
Llinas, R., New York University School of Medicine, New York: Ion channels and integration.
Mackinnon, R., The Rockefeller University, New York: Structure and function of voltage-gated channels.
Nerbonne, J., Washington University School of Medicine, St. Louis, Missouri: Voltage-gated K channels in heart and neurons.
Nolan, M., Columbia University, New York: Hyperpolarization-activated cation channels.
Otis, T., University of California, Los Angeles: Glutamate uptake.
Raman, I., Northwestern University, Evanston, Illinois: Action potential, Hodgkin-Huxley, Na channels.
Svoboda, K., Cold Spring Harbor Laboratory: Imaging methods for studying ion channels.
Trimmer, J., Stony Brook University, New York: Accessory subunits of voltage-gated channels.
Turrigiano, G., Brandeis University, Waltham, Massachusetts: Homeostatic synaptic plasticity.
Zamponi, G., University of Calgary, Canada: Voltage-gated Ca channels.

Making and Using DNA Microarrays

June 17–24

INSTRUCTORS **V. Iyer,** University of Texas, Austin
J. Lieb, University of North Carolina, Chapel Hill

ASSISTANTS **E. Diaz,** University of California, Berkeley, Davis
A. Carroll, University of California, San Francisco
K. Jeffers, University of Texas, Austin
P. Killion, University of Texas, Austin
C. Lee, University of North Carolina, Chapel Hill
M. Llinas, University of California, San Francisco
D. Verdnik, Axon Instruments, Foster City, California

A DNA microarray is a simple, inexpensive, and versatile tool for experimental explorations of genome structure, gene expression programs, gene function, and cell and organismal biology. In this hands-on course, students were guided through the process of building a robot for printing DNA microarrays, preparing DNA samples and slides to be used for printing microarrays, printing DNA microarrays, designing and conducting experiments for analysis by DNA microarray hybridization, data analysis, display, and interpretation. Experimental applications covered in the course included systematic studies of global gene expression programs, inferring gene function using microarrays, genotyping, and mea-

suring changes in gene copy number. Students who completed this course were able to set up their own independent facility for printing and experimental use of DNA microarrays. Guest lecturers presented on state-of-the-art technology, experimental applications, and interpretation of large genomic data sets.

PARTICIPANTS

Agalioti, T., Ph.D., Columbia University, New York
Agindotan, B., Ph.D., University of Idaho, Moscow
del Pilar Aguinaga, M., Ph.D., Meharry Medical College, Nashville, Tenneessee
Alexeyev, M., Ph.D., University of South Alabama, Mobile
Baxi, Mohit, Ph.D., Canadian Food Inspection Agency, Alberta, Canada
Constancia, J., Ph.D., The Babraham Institute, Cambridge, United Kingdom
Corley-Smith, G., Ph.D., Oregon State University, Corvallis
Englen, M., Ph.D., U.S. Department of Agriculture, Athens, Georgia
Fong, S., Ph.D., California Pacific Medical Center, San Francisco
Gaitan-Solis, E., B.A., Centro Internacional de Agricultura Tropical, Cali, Columbia
Hanson, L., Ph.D., Mississippi State University
Levenson, C., Ph.D., Florida State University, Tallahassee
Li, H., B.S., University of California, Los Angeles
Liebana, E., Ph.D., Veterinary Laboratories Agency-Weybridge, Addelstone, United Kingdom
Pearce, D., Ph.D., British Antarctic Survey, Cambridge, United Kingdom
Pessler, F., Ph.D., The Children's Hospital of Philadelphia, Pennsylvania
Ranade, S., Ph.D., Harvard Medical School, Boston, Massachusetts
Trolliet, M., Ph.D., Boston University School of Medicine, Massachusetts
Yennu-Nanda, V., Ph.D., University of Texas/M.D. Anderson Cancer Center, Houston
York, D., M.S., University of Delaware Biotechnology Institute, Newark

SEMINARS

Dudoit, S., University of California, Berkeley: The bioconductor project: Open-source statistical software for the analysis of microarray data.
Murphy, C., University of California, San Francisco: Using microarrays to find *C. elegans* aging genes.
Sherlock, G., Stanford University, California: Organization and analysis of DNA microarray data.
Skene, P., Duke University, Durham, North Carolina: Old dogs and microarrays—Bringing microarrays into an established research program.

Developmental Neurobiology

June 20–July 3

INSTRUCTORS **B. Barres,** Stanford University School of Medicine, California
J. Sanes, Washington University School of Medicine, St. Louis, Missouri

The aim of this lecture course was to discuss principles and recent advances in developmental neurobiology. Major topics considered included determination, proliferation and differentiation of neural cells; trophic interactions in neural development; guidance of axons to targets; formation and rearrangement of synapses; activity-dependent synaptic remodeling and development of behavior. These topics were considered within the context of the development of both invertebrate and vertebrate neural systems. Prospective students had a background in neurobiology or molecular biology. The course was held at the Laboratory's Banbury Conference Center.

PARTICIPANTS

Andersson, E., M.S., Lund University, Sweden
Arikkath, J., Ph.D., University of California, San Francisco
Campbell, G., Ph.D., University of California, Berkeley
Choe, K., Ph.D., Stanford University School of Medicine, California
Daneman, R., B.S., Stanford University, California
Deppmann, C., B.S., Johns Hopkins University School of Medicine, Baltimore, Maryland
Gelkop-Abramowicz, S., Ph.D., Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada
Hafidadottir, B., M.S., Harvard Medical School, Boston, Massachusetts

Huberman, A., M.A., University of California, Davis
Leung, K., B.S., University of Cambridge, United Kingdom
Karpova, A., Ph.D., Cold Spring Harbor Laboratory
Meletis, K., M.S., Karolinska Institutet, Stockholm, Sweden
Nielsen, M., B.S., University of Copenhagen, Denmark
Pak, W., B.S., The Salk Institute, La Jolla, California
Pham, K., Ph.D., Stanford University School of Medicine, California
Raveh, T., Ph.D., Stanford University, California
Reis, R., Ph.D., Oxford University, Oxford, United Kingdom
Thompson, C., M.A., Harvard Medical School, Boston, Massachusetts
Torborg, C., B.A., University of California, San Diego
Valente, A., B.A., Gulbenkian Institute of Science, Oeiras, Portugal
Vallstedt, A., M.S., Karolinska Institutet, Stockholm, Sweden
van den Akker, W., Ph.D., Netherlands Institute for Developmental Biology, Utrecht, The Netherlands
Wu, J., Ph.D., Stanford University, California

SEMINARS

Barres, B., Stanford University School of Medicine, California: Trophism. GLIA.
Cline, H., Cold Spring Harbor Laboratory: Axonal arborization.
Davis, G., University of California, San Francisco: Synapse growth.
Dulac, C., Harvard University, Cambridge, Massachusetts: Transgenic approaches to behavior.
Feldman, D., University of California, San Diego: Plasticity in cortex.
Ghosh, A., Johns Hopkins Medical School, Baltimore, Maryland: Dendrite guidance.
Jan, Y.N., University of California, San Francisco: Neurogenesis and neuronal differentiation.
Jessell, T., Columbia University, New York: Regionalization and patterning.
Knudsen, E., Stanford University School of Medicine, California: Auditory learning and plasticity.
Lichtman, J., Washington University, St. Louis, Missouri: Synaptic rearrangements.
Morrison, S., University of Michigan, Ann Arbor: Lineage and stem cells.
O'Leary, D., Salk Institute, San Diego, California: Cortex-patterning. Map formation in brain.
Raff, M., University College London, United Kingdom: Cell survival and growth.
Sanes, J., Washington University School of Medicine, St. Louis, Missouri: Overview of neural development. Synapse formation. Synaptic specificity.
Stern, C., University College London, United Kingdom: Neural induction.
Tessier-Lavigne, Stanford University, California: Axon guidance and regeneration.
Van Vactor, D., Harvard Medical School, Boston, Massachusetts: Axon growth.
Walsh, C., Beth Israel Deaconess Medical Center, Boston, Massachusetts: Neuronal migration and its disorders.

Mouse Behavioral Analysis

June 27–July 10

INSTRUCTORS **S. Anagostaras,** Emory University, Atlanta, Georgia
M. Mayford, The Scripps Research Institute, La Jolla, California

CO-INSTRUCTOR **M. Fanselow,** University of California, Los Angeles

ASSISTANTS **S. Mitchell,** Emory University, Atlanta, Georgia
J. Quinn, University of California, Los Angeles
M. Tinsley, University of California, Los Angeles
A. Wilson, Emory University, Atlanta, Georgia

This course was intended to provide a theoretical and experimental introduction to behavioral analysis in the mouse, with a focus on learning and memory. It was specially designed for geneticists, molecular biologists, pharmacologists, and electrophysiologists with a need for a hands-on introduction to behavioral analysis of the mouse. Additionally, the course covered the principles of using mutant mice in behavioral studies, as well as the issues involved in integrating behavioral, neuroanatomical, neurophysiological, and molecular findings. Among the methods presented were the water maze, cued and contextual fear conditioning, natural ethologically-relevant learning, open field behavior, the rotor-rod, and other activity tests.

PARTICIPANTS

Adachi, N., B.S., Kobe University, Kobe, Japan
Bryceson, Y., M.S., NIH/Karolinska Institutet, Rockville, Maryland
Deisseroth, K., Ph.D., Stanford University, California
Diana, G., M.D., Higher Institute of Health, Rome, Italy
Fischer, D., Ph.D., Netherlands Institute for Brain Research, Amsterdam, The Netherlands
Hertz, J., B.S. Massachusetts Institute of Technology, Cambridge
Hossain, S., Ph.D., Centre for Molecular Medicine & Therpaeutics, British Columbia, Canada
Iarkov, A., Ph.D., Sun Health Research Institute, Sun City, Arizona
Kanisek, M., B.S., Mount Sinai Hospital, Toronto, Canada
Singh, M., Ph.D., Vanderbilt University, Nashville, Tennessee

SEMINARS

Bourtchouladze, R., Helicon Therapeutics, Inc., Woodbury, New York: cAMP signaling and memory: From genes to drugs.
Costa, R., Duke University, Durham, North Carolina: Cognitive dysfunction: From molecules to systems in mouse models.
Holland, P., Johns Hopkins University, Baltimore, Maryland: Introduction to learning and memory.
Knowlton, B., University of California, Los Angeles: The hippocampus and human memory.
Maren, S., University of Michigan, Ann Arbor: Fear memory circuits in the brain.
Murphy, G., University of California, Los Angeles: Mouse models of age-related cognitive decline.
Thomas, M., University of Minnesota, Minneapolis: Psychostimulant-induced plasticity in neural circuitry and behavior.
Waldman, I., Emory University, Atlanta, Georgia: Dopamine genes and children's disruptive behavior disorders.

Arabidopsis Molecular Genetics

June 27–July 17

INSTRUCTORS **J. Bowman,** University of California, Davis
U. Grossniklaus, University of Zurich, Switzerland
R. Pruitt, Purdue University, W. Lafayette, Indiana

ASSISTANTS **A. Izhaki,** University of California, Davis
R. Lee, Purdue University, W. Lafayette, Indiana
J.M.E. Restrepo, University of Zurich, Switzerland

This course provided an intensive overview of topics in plant growth, physiology, and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from *Arabidopsis thaliana* and other model plants and provided an introduction to current methods used in *Arabidopsis* research. It was designed for scientists with experience in molecular techniques or in plant biology who wish to work with *Arabidopsis*. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Discussions of important topics in plant research were presented by the instructors and by invited speakers. These seminars included plant morphology and anatomy; plant development (including development of flowers, roots, meristems, and leaves, male and female gametophytes, and embryos); perception of light and photomorphogenesis; and synthesis, function, and perception of hormones. Lectures describing bioinformatics tools available to the *Arabidopsis* community, and the resources provided by the *Arabidopsis* genome project to accelerating *Arabidopsis* research were also included. Speakers provided overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions provided an introduction to

important techniques currently used in *Arabidopsis* research. These included studies of *Arabidopsis* development, mutant analysis, in situ detection of RNA, histochemical staining, transient gene expression, applications of green fluorescent protein fusions, protein interaction and detection, proteomics approaches, transcription profiling, techniques commonly used in genetic and physical mapping. The course also included several short workshops on important themes in genetics.

This year's speakers included Rick Amasino, Jody Banks, Kathryn Barton, David Baum, Winslow Briggs, James Carrington, Dean DellaPenna, Nancy Dengler, Hugo Dooner, Ted Farmer, Roger Hangarter, Jeff Harper, Thomas Jack, David Jackson, Nancy Kerk, Maarten Koornneef, Hong Ma, Jocelyn Malamy, Ashi Malekafzali, Robert Martienssen, Peter McCourt, Tim McNellis, June Nasrallah, Scott Peck, Leonore Reiser, Eric Richards, G. Eric Schaller, and Ian Sussex.

PARTICIPANTS

Bierwagen, T., B.S., Iowa State University, Ames
Blum, E., M.S., Weizmann Institute of Science, Rehovot, Israel
Bouveret, R., M.S., Swiss Federal Institute of Technology, Zurich, Switzerland
Eren, E., M.S., Worcester Polytechnic Institute, Massachusetts
Galego, L., Ph.D., Instituto de Tecnologia Quimica e Biologica Oeiras, Portugal
Hansen, B., M.S., Royal Veterinary and Agricultural and University, Copenhagen, Denmark
Khambatta, Z., M.S., Bali Helix, W. Chicago, Illinois
Martelotto, L., B.S., Institute for Molecular and Cellular Biology of Rosario, Argentina
McLean, M., Ph.D., University of Guelph, Ontario, Canada
Melo, D., B.S., Performance Plants Inc., Ontario, Canada
Moreau, C., B.S., Umea Plant Science Centre, Sweden
Oono, Y., B.S., RIKEN Tsukuba Institute, Ibaraki, Japan
Sato, M., Ph.D., Hokkaido University, Sapporo, Japan
Sohn, S.O., B.S., Chonnam National University, Gwangju, South Korea
Somberg, A.M., M.S., Swedish University of Agricultural Sciences, Uppsala, Sweden
Teixeira, R., B.S., SLU-Swedish University of Agricultural Sciences, Uppsala, Sweden

SEMINARS

Amasino, R., University of Wisconsin, Madison: Flowering time.
Banks, J., Purdue University, W. Lafayette, Indiana: Gametophytes and evolution.
Barton, K., Carnegie Institution of Washington, Stanford, California: Embryogenesis.
Baum, D., University of Wisconsin, Madison: Evolution.
Bowman, J., University of California, Davis: Leaves.
Briggs, W., Carnegie Institution of Washington, Stanford, California: The big picture.
Carrington, J., Oregon State University, Corvallis: Epigenetics I.
DellaPenna, D., Michigan State University, E. Lansing: Secondary metabolism.
Dengler, N., University of Toronto, Ontario, Canada: Plant anatomy.
Dooner, H., Rutgers University, Piscataway, New Jersey: Transposons.
Farmer, T., University of Lausanne, Switzerland: Custom microarrays.
Kerk, N., Yale University, Guilford, Connecticut: Laser capture microscopy.
Grossniklaus, U., University of Zurich, Switzerland: Female gametophyte.
Hangarter, R., Indiana University, Bloomington: Light responses.
Harper, J., The Scripps Research Institute, La Jolla, California: Calcium and signaling.
Koornneef, M., Wageningen University, The Netherlands: Quantitative genetics.
Malamy, J., University of Chicago, Illinois: Roots.
Nasrallah, J., Cornell University, Ithaca, New York: Self-incompatibility.
Pruitt, B., Purdue University, W. Lafayette, Indiana: Male gametophyte and signaling.
Sussex, I., Yale University, Guilford, Connecticut: Introduction.
Jack, T., Dartmouth College, Hanover, New Hampshire: Flowers.
Jackson, D., Cold Spring Harbor Laboratory: Meristem and leaf.
Ma, H., Pennsylvania State University, University Park: Anther development and meiosis.
Martienssen, R., Cold Spring Harbor Laboratory: Enhancer traps.
McCourt, P., University of Toronto, Ontario, Canada: Hormone networks.
McNellis, T., Pennsylvania State University, Philadelphia: Plant pathogen.
Peck, S., Sainsbury Laboratory, Norwich, United Kingdom: Proteomics.
Reiser, L., The *Arabidopsis* Information Resource, Stanford, California: Bioinformatic resources.
Richards, E., Washington University, St. Louis, Missouri: Epigenetics II.
Schaller, G.E., University of New Hampshire, Durham: Ethylene.

Neurobiology of *Drosophila*

June 27–July 17

INSTRUCTORS **R. Baines,** University of Warwick, Coventry, United Kingdom
S. de Belle, University of Nevada, Las Vegas
D. Van Vactor, Harvard Medical School, Boston, Massachusetts

ASSISTANTS **K. Baughman,** Harvard Medical School, Boston, Massachusetts
A. Belay, University of Toronto, Mississauga, Canada
E. Pym, University of Warwick, Coventry, United Kingdom

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students through established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The three-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that were used in the investigation of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques including embryo in situ hybridization; labeling of identified neurons; electrophysiological recording from

nerves and muscles; and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system. This year's lecturers included: Vanessa Auld, Ross Cagan, Josh Dubnau, Daniel Eberl, James Gergel, Tanja Godenschwege, Andreas Keller, Juergen Knoblich, Matthias Landgraf, Dennis Mathew, Rod Murphey, Howard Nash, Kevin O'Dell, Dietmar Schmucker, Marla Sokolowski, Bryan Stewart, Roland Strauss, Ulrich Tepass, and Jennifer Shuler Waters.

PARTICIPANTS

Ball, R., B.S., University of California, Berkeley
Besse, F., Ph.D., European Molecular Biology Laboratory, Heidelberg, Germany
Bhalerao, S., M.S., Research Institute of Molecular Pathology, Vienna, Austria
Bolduc, F., M.D., McGill University, Montreal, Canada
Dimitropoulou, A., B.A., University of Miami School of Medicine, Florida
Kaun, K., B.S., University of Toronto, Mississauga, Canada
Kristiansen, L., M.S., University of Michigan, Ann Arbor
Muraro, N., M.S., University Warwick, Coventry, United Kingdom
Schmidt, M., M.A., Max-Planck-Institut, Göttingen, Germany
Schulz, J., M.D., University of Leuven, Belgium
Thimgan, M., B.S., University of North Carolina, Chapel Hill
Uthaman, S., Ph.D., University of Massachusetts, Amherst

SEMINARS

Auld, V., University of British Columbia, Vancouver, Canada: Glial development.
Baines, R., University of Warwick, United Kingdom: Synaptic plasticity. CNS Electrophysiology.
Budnick, V., University of Massachusetts, Amherst: The synapse.
Cagan, R., Washington University, St. Louis, Missouri: Death and the eye.
de Belle, S., University of Nevada, Las Vegas: Introduction to fly behavior. Learning and memory: Hardware.
Dubnau, J., Cold Spring Harbor Laboratory: Learning and memory: Software.
Eberl, D., University of Iowa, Iowa City: Mechanosensation.
Gergel, J., Cold Spring Harbor Laboratory: Genomics 101. Genomics 102.
Keller, A., The Rockefeller University, New York: Sensory physiology.
Landgraf, M., Cambridge University, United Kingdom: Neuronal identity.
Murphy, R., University of Massachusetts, Amherst: Giant fiber system.
Nash, H., NIMH, National Institutes of Health, Bethesda, Maryland: Anesthesia.
O'Dell, K., University of Glasgow, United Kingdom: Courtship.
Schmucker, D., Harvard University, Cambridge, Massachusetts: Postembryonic PNS.
Shuler Waters, J., Harvard University, Cambridge, Massachusetts: Confocal microscopy.
Sokolowski, M., University of Toronto, Canada: Foraging.
Stewart, B., University of Toronto, Canada: Electrophysiology 101. Synaptic transmission.
Strauss, R., Theodor-Bovri-Institute, Wurzburg, Germany: Motor behavior. Visual system.
Tepass, U., University of Toronto, Ontario, Canada: The fly embryo and adult.
Van Vactor, D., Harvard University, Cambridge, Massachusetts: Fly genetics 101. Axon guidance.

Advanced Techniques in Molecular Neuroscience

July 2–17

INSTRUCTORS
J. Boulter, University of California, Los Angeles
L. Henry, Cold Spring Harbor Laboratory
K. Jensen, The Rockefeller University, New York
C. Lai, Scripps Research Institute, La Jolla, California
D. Lavery, Purdue Pharma LP, Cranbury, New Jersey

ASSISTANTS
J. Arjomand, University of California, Los Angeles
I. Cheung, Scripps Research Institute, La Jolla, California
K. Dredge, The Rockfeller University, New York
A. Klaassen, University of California, Los Angeles
M. Samson, Harvard Medical School/HHMI, Boston, Massachusetts
W. Walwyn, University of California, Los Angeles

This newly revised laboratory and lecture course was designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the most advanced techniques in molecular neuroscience. The course curriculum was divided into three sections: an extensive and up-to-date set of laboratory exercises; daily lectures covering the theoretical and practical aspects of the various methods used in the laboratory; and a series of evening research seminars. The informal and interactive evening lectures were given by leading molecular neuroscientists and illustrated the ways in which the various experimental approaches have been used to advance specific areas of neurobiology. In this

year's course, the laboratory portion included topics such as an introduction to the design and use of animal virus vectors in neurobiology; the use of small interfering RNAs (siRNA) for regulating the expression of specific genes in neurons; practical exercises in gene delivery systems, including mammalian cell transfection protocols and single-cell electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use, and design of bacterial artificial chromosome (BAC) transgenic vectors; multiplex and whole-genome expression analyses using the most recent DNA microarray technologies (including labeled probe preparation, data analyses, mining, and interpretation); quantitative real-time reverse transciptase–polymerase chain reaction (RT-PCR) analyses from small numbers of cells (RNA purification, PCR optimization, interpretation of results); single-cell PCR and cDNA library construction; and methods and application of RNA amplification (aRNA). Each laboratory module was followed by comprehensive data analyses and interpretation, protocol troubleshooting, and suggestions for ways to improve or modify the existing technique. Finally, course participants were introduced to bioinformatics and a wide range of Internet resources which are available to molecular neuroscientists.

PARTICIPANTS

Akerman, C., Ph.D., Cold Spring Harbor Laboratory
Baker, C., Ph.D., University of Cambridge, United Kingdom
Chu, J., Ph.D., The Scripps Research Institute, La Jolla, California
Close, J., B.A., University of Washington, Seattle
Duan, J., Ph.D., University of Chicago, Illinois
Engstrom, L., M.S., Linkoping University, Sweden
Jakobs, T., M.D., Massachusetts General Hospital, Boston
Jolly, R., M.S., University College London, United Kingdom
Ju, C., B.S., Columbia University, New York
Lambe, E., Ph.D., Yale University, New Haven, Connecticut
Lopez-Bendito, G., Ph.D., University of Oxford, United Kingdom
Lukaszewicz, A., M.S., INSERM, Bron, France
McQuiston, R., Ph.D., Virginia Commonwealth University Medical Campus, Richmond
Ting, J., B.S., University of Washington, Seattle
Watkins, T., A.B., Stanford University School of Medicine, California
Weiner, J., Ph.D., Washington University School of Medicine, St. Louis, Missouri

SEMINARS

Barres, B., Stanford University, California: Neuron-glial interactions in the developing CNS.
Eberwine, J., University of Pennsylvania, Philadelphia: Molecular analysis of dendritic functioning: mRNA localization, mRNA transport, and local protein synthesis.
Feinstein, P., The Rockefeller University, New York: Axon guidance with odorant receptors.
Fishell, G., Skirball Institute, New York University, New York: Experimental genetic and cell biological approaches to patterning, proliferation, and migration in the developing telencephalon.
Patapoutian, A., The Scripps Institute, La Jolla, California: The sense of touch: A genomic approach.
Scrable, H., University of Virginia, Charlottesville: Conditional doesn't have to be forever: Reversible control of gene expression in the mouse with the *lac* operator-repressor system.
Weinmaster, G., David Geffen School of Medicine at the University of California, Los Angeles: Modulation of ligand-induced notch signaling.
Yang, W., David Geffen School of Medicine at the University of California, Los Angeles: BAC transgenic approach to study neuronal function and dysfunction.

WORKSHOP ON THEORETICAL NEUROSCIENCE
Optimization and Constraints in the Evolution of Brain Design

July 14–20

INSTRUCTORS **D. Chklovskii,** Cold Spring Harbor Laboratory
C. Stevens, The Salk Institute

In this workshop, participants presented successful examples of constrained optimization of brain design and function and debated the virtues and pitfalls of the optimization approach. The brain is a result of evolution by natural selection, which favors optimal use of limited resources; it is natural to use constrained optimization as a tool to understand brain design and function. An understanding of what is being optimized and under which constraints would allow us to unify many disjointed experimental facts into a single theoretical framework. Such framework can yield a deeper understanding of the brain and can guide future experiments.

LECTURERS

Andreau, A., Johns Hopkins University, Baltimore, Maryland: Optimization of sensory information processing.
Balasubramanian, V., University of Pennsylvania, Philadelphia: Why the retina uses parallel channels II.
Bialek, W., Princeton University, New Jersey: Optimization in neurobiology.
Boahen, K., University of Pennsylvania, Philadelphia: Wiring feature maps by following gradients.
Condron, B., University of Virginia, Charlottesville: Wiring of serotonergic neurons in development.
Dimitrov, A., University of Montana, Bozeman: Unit failure and neural circuit complexity.
Koulakov, A., University of Utah, Salt Lake City: How to play golf with eye balls.
Levy, W., University of Virginia, Charlottesville: Energy-efficient neuronal computation.
Miller, K., University of California, San Francisco: Model for contrast-invariant orientation tuning in V1.
Mitra, P., Cold Spring Harbor Laboratory, New York: Optical fiber capacity.
Mokhtarzada, Z., University of Maryland, College Park: Optimal wiring in neuroanatomy.
Purves, D., Duke University. Chapel Hill, North Carolina: What do music and vision have in common?
Shefi, O., Tel-Aviv University, Israel: Biophysical constraints on neuronal branching.
Stepanyants, A., Cold Spring Harbor Laboratory, New York: Branching law of axons.
Sterling, P., University of Pennsylvania, Philadelphia: Why the retina uses parallel channels I.
Wang, S., Princeton University, New Jersey: Speed limits in mammalian brains: Scaling constraints from biophysics.
White, J., Boston University, Massachusetts: Minimal axon diameter.

PARTICIPANTS

Atwal, G., Princeton University, New Jersey
Buchanan, D., Columbia University, New York
Chigirev, D., Princeton University, New Jersey
Goldberg, D., Johns Hopkins University, Baltimore, Maryland
Howe, C., Duke University, Durham, North Carolina
Mysore, S., California Institute of Technology, Pasadena
Nundy, S., Duke University, Durham, North Carolina
Schneider, K., Princeton University, New Jersey
Snider, J., University of California, Irvine
Taba, B.,University of Pennsylvania, Philadelphia
Wang, J., Stony Brook University, New York
Wyatt, K., Princeton University, New Jersey
Yang, Z., Duke University, Durham, North Carolina

C. elegans

July 22–August 11

INSTRUCTORS **B. Bowerman,** University of Oregon, Eugene
A. Chisholm, University of California, Santa Cruz
S. Clark, New York University School of Medicine, New York

ASSISTANTS **C. Carter,** University of Oregon, Eugene
S. Moseley, University of California, Santa Cruz
W. Woo, University of California, Santa Cruz

This course was designed to familiarize investigators with *C. elegans* as an experimental system, with an emphasis on both classical genetic analysis and reverse genetic approaches. A major goal was to teach students how to successfully exploit the information generated by the *C. elegans* Genome Project. The course was suited both for those who have a current training in molecular biology and

some knowledge of genetics, but had no experience with *C. elegans*, and for students with some prior worm experience who wished to expand their repertoire of expertise. The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction and screening of deletion libraries, and RNA inactivation. The course was designed to impart sufficient training to students in the most important attributes of the *C. elegans* system to enable students to embark on their own research projects after returning to their home institutions.

PARTICIPANTS

Berkovitz, H., Ph.D., Merrimack College, Andover, Massachusetts
Conner, S., Ph.D., The Scripps Research Institute, La Jolla, California
Cueva, J., Ph.D., Stanford University, California
Furukawa, T., Ph.D., Osaka Bioscience Institute, Osaka, Japan
Garvis, S., Ph.D., Centre National de Recherche Scientific, Marseille, Franc
Glaser, S., B.S., Eidgenössiche Technische Hochschule (ETH) Hoenggerberg, Zurich, Switzerland
Handwerger, K., Ph.D., Whitehead Institute, Cambridge, Massachusetts
Jenna, S., Ph.D., McGill University, Montreal, Canada
Liakopoulos, D., Ph.D., ETH Hoenggerberg, Zurich, Switzerland
Lim, R., Ph.D., Whitehead Institute, Cambridge, Massachusetts
Miranda-Vizuete, A., Ph.D., Karolinska Institutet, Huddinge, Sweden
Raman, C.S., Ph.D., University of Texas, Houston
Rhoads, R., Ph.D., Louisiana State University Health Sciences Center, Shreveport
Sasano, Y., B.S., Kobe University, Hyogo, Japan
Taylor, R., Ph.D., University of Dublin, Ireland
Toyoshima, F., Ph.D., Kyoto University, Japan

SEMINARS

Ambros, V., Dartmouth Medical School, Hanover, New Hampshire: Small RNAs in *C. elegans*.
Conradt, B., Max-Planck-Institute of Neurobiology, Martinsried/Munich, Germany: *C. elegans* neurobiology.
Felix, M.-A., L'Institut Jacques Monod, Paris, France: Genetic studies of nematode evolution.
Goldstein, B., University of North Carolina, Chapel Hill: Cell polarity in *C. elegans* embryos.
Hall, D., Albert Einstein School of Medicine, Bronx, New York: *C. elegans* anatomy.
Harris, T., Cold Spring Harbor Laboratory: Wormbase and *C. briggsae*.
Jin, Y., University of California, Santa Cruz: *C. elegans* neurobiology.
Mori, I., Nagoya University, Nagoya, Japan: *C. elegans* neurobiology.
Priess, J., Fred Hutchinson Cancer Research Center, Seattle, Washington: Cell fate patterning in early *C. elegans* embryos.
Rose, A., University of British Columbia, Vancouver, Canada: Essential genes in *C. elegans*.
Sengupta, P., Brandeis University, Waltham, Massachusetts: *C. elegans* neurobiology.
White, J., University of Wisconsin, Madison: Cytokinesis in *C. elegans* embryos.
Zarkower, D., University of Minnesota, Minneapolis: Sex determination in *C. elegans*.

Eukaryotic Gene Expression

July 22–August 11

INSTRUCTORS **L. Attardi,** Stanford University, California
W.L. Kraus, Cornell University, Ithaca, New York
M. Timmers, University Medical Centre, Utrecht, The Netherlands

ASSISTANTS **S. Jacobs,** Stanford University, California
M.Y. Kim, Cornell University, Ithaca, New York
M. Klejman, University Medical Centre, Utrecht, The Netherlands

This course was designed for students, postdocs, and principal investigators who have recently ventured into the exciting area of gene regulation. The course focused on state-of-the-art strategies and techniques employed in the field. Emphasis was placed on both in vitro and in vivo protein-DNA interactions and on novel methodologies to study gene regulation.

Students made nuclear extracts, performed in vitro transcription reactions, and measured RNA levels using primer extension. Characterization of the DNA-binding properties of site-specific transcription factors was carried out using electrophoretic mobility-shift and DNase I footprinting assays. In addition, students learned techniques for the assembly and analysis of chromatin in vitro. This included transcription assays, chromatin footprinting, and chromatin remodeling assays.

During the past few years, the gene regulation field has developed in vivo approaches to study gene regulation. Students were exposed to the chromatin immunoprecipitation technique. They also used RNA interference for specific knockdown experiments in mammalian cells. In addition, determining cellular gene expression profiles has been accelerated tremendously by DNA microarray technology. Students received hands-on training in performing and interpreting results from DNA microarrays. Experience with basic recombinant DNA techniques was a prerequisite for admission to this course. Lectures by the instructors covered the current status of the gene expression field, theoretical aspects of the methodology, and broader issues regarding strategies for investigating the regulation of gene expression in eukaryotes.

Guest lecturers discussed contemporary problems in eukaryotic gene regulation and technical approaches to their solution. The speakers this year included: David Bentley, Shelley Berger, Arnold Berk, David Botstein, Brian Dynlacht, Barbara Graves, Gregory Hannon, Jacqueline Lees, Anders Naar, Ali Shilatifard, Laszlo Tora, Peter Verrijzer, and Keith Yamamoto.

PARTICIPANTS

Abdel-Fattah, G., Ph.D., Baylor College of Medicine, Houston, Texas
Bacanamwo, M., Ph.D., Morehouse School of Medicine, Atlanta, Georgia
Bansal, P., M.S., University of Pittsburgh, Pennsylvania
Civitareale, D., B.S., Italian National Council of Research, Rome
De la Mata, M., M.S., Laboratorio de Fisiologia y Biologia Molecular, Buenos Aires, Argentina
Izumo, M., Ph.D., Vanderbilt University, Nashville, Tennessee
Kulbokas, E., B.A., Massachusetts Institute of Technology/ Whitehead Institute, Cambridge
Li, S., M.S., Marquette University, Milwaukee, Wisconsin
Navarro Gil, P., M.S., Institut Pasteur, Paris, France
Pagano, A., Ph.D., University of Genoa, Italy
Pallafacchina, G., Ph.D., University of Padova, Italy
Parlakian, A., M.A., University Paris 7, France
Prokunina, L., M.S., Uppsala University, Sweden
Ragvin, A., M.S., University of Bergen, Norway
Schuster, M., M.S., Copenhagen University Hospital, Denmark
Van Laere, A., Ph.D., Swedish University of Agricultural Sciences, Uppsala, Sweden

SEMINARS

Attardi, L., Stanford University, California: Using mouse models to understand *p53*.
Bentley, D., University of Colorado Health Science Center, Denver: Integration of transcription and pre-mRNA processing.
Berger, S., Wistar Institute, Philadelphia, Pennsylvania: Histone and factor modifications in gene activation.
Berk, A., University of California, Los Angeles: How does the interaction between an activation domain and the mediator complex stimulate transcription?
Botstein, D., Princeton University, New Jersey: Studies of genome-wide expression in cancer.
Dynlacht, B., New York University Cancer Institute, New York: Transcriptional control of the cell cycle.
Graves, B., University of Utah, Salt Lake City: Strategies for specificity among ETS family of transcription factors.
Hannon, G., Cold Spring Harbor Laboratory: RNAi and its application.
Kraus, L., Cornell University, Ithaca, New York: Molecular mechanisms of estrogen-dependent transcription.
Lees, J., Massachusetts Institute of Technology Center for Cancer Research, Cambridge: Understanding E2F transcription factor function in vivo.
Naar, A., Massachusetts General Hospital Cancer Center/Harvard Medical School, Charlestown: Functional and structural characterization of the human ARC/Mediator transcriptonal coactivator complex.
Shilatifard, A., St. Louis University, Missouri: Biochemical and proteomic methods in defining molecular mechanisms of gene expression by RNA polymerase II.
Timmers, M., University Medical Centre, Utrecht, The Netherlands: Dynamics in RNA polymerase II transcription.
Tora, L., Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France: Gene-specific transcriptional regulation by the diversity in the components of general transcription factors.
Verrijzer, P., Leiden University Medical Centre, The Netherlands: Chromatin regulation of development and cell proliferation.
Yamamoto, K., University of California, San Francisco: Functional probing of transcriptional regulatory complexes.

Imaging Structure and Function in the Nervous System

July 22–August 11

INSTRUCTORS
F. Helmchen, Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany
D. Kleinfeld, University of California San Diego, La Jolla
T. Murphy, University of British Columbia, Vancouver, Canada

ASSISTANTS
T. Nevian, Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany
Y. Otsu, University of British Columbia, Vancouver, Canada
P. Tsai, University of California, San Diego
W. Tyler, Harvard University, Cambridge, Massachusetts
J. Waters, Max-Planck-Institut, Heidelberg, Germany
S. Zhang, University of British Columbia, Vancouver, Canada

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensified laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser-scanning systems, function-

al fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multiphoton laser-scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. We used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells. Lecturers included Wolfhard Almers, William Betz, Holly Cline, Winfried Denk, Graham Ellis-Davies, Jamil Farbes, Joseph Fetcho, Kurt Haas, Ernst Keller, Fred Lanni, Jeff Lichtman, Jerome Mertz, Gero Miesenboeck, Anneliese Schaefer, Gordon Shepherd, Karel Svoboda, Philbert Tsai, and Roger Tsien.

PARTICIPANTS

Barbour, D., Ph.D., Johns Hopkins School of Medicine, Baltimore, Maryland
Cruz, H., M.S., University of Geneva, Switzerland
Gao, W., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Haggarty, S., B.S., Harvard University, Cambridge, Massachusetts
Humeau, Y., Ph.D, University of Basel, Switzerland
Kara, P., Ph.D., Harvard Medical School, Boston, Massachusetts
Lindskog, M., Ph.D., Stanford University, California
Ringstad, N., Ph.D., Massachusetts Institute of Technology, Cambridge
Roth-Alpermann, C., M.S., Max-Planck-Institute of Neurobiology, Muenchen-Martinsried, Germany
Rumpal, N., M.S., University at Albany, New York
Sorensen, L., M.S., Karolinska Institutet, Huddinge, Sweden
Wadiche, J., B.A., Vollum Institute, Portland, Oregon

SEMINARS

Almers, W., Oregon Health & Science University, Portland: Vesicles and TIRF microscopy.
Betz, B., University of Colorado Medical School, Denver: Imaging secretion, FM-1-43.
Cline, H., Cold Spring Harbor Laboratory: In vivo imaging in tadpoles.
Denk, W., Max-Planck-Institute for Medical Research, Heidelberg, Germany: Multiphoton free lecture.
Ellis-Davies, G., MCP Hahnemann University, Philadelphia, Pennsylvania: Ca and transmitter uncaging.
Fetcho, J., Stony Brook University, New York: In vivo imaging in zebrafish.
Helmchen, F., Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany: Ca imaging basics, ratioing. Applications of 2-photon microscopy.
Keller, E., Carl Zeiss, Inc., Thornwood, New York: Basic microscopy.
Kleinfeld, D., University of California, San Diego, and Tsai, P., University of California, San Diego: Basic optics. Digital image processing.
Haas, K., Cold Spring Harbor Laboratory: Single-cell electroporation.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Fluorescence microscopy.
Lichtman, J., Washington University, St. Louis, Missouri: Confocal microscopy.
Mertz, J., ESPCI, Paris, France: Nonlinear microscopies
Miesenboeck, G., Memorial Sloan-Kettering Cancer Center, New York: GFP probes to image exocytosis.
Murphy, T., University of British Columbia, Vancouver, Canada: Calcium imaging applications.
Shepherd, G., Cold Spring Harbor Laboratory: Glutamate uncaging.
Sullivan, R., Hamamatsu Photonic Systems, Bridgewater, New Jersey: CCD cameras intro.
Svoboda, K., Cold Spring Harbor Laboratory: 2-photon microscopy. Applications of 2-photon microscopy.
Tsai, P., University of California, San Diego: Nonlinear optics for imaging and ablation.
Tsien, R., University of California, San Diego: Bapta and synthetic Ca^{2+} indicators. Imaging intracellular signaling.

Yeast Genetics

July 22–August 11

INSTRUCTORS **D. Amberg,** SUNY at Syracuse, Health Sciences Center, New York
D. Burke, University of Virginia, Charlottesville
J. Strathern, National Cancer Institute, Frederick, Maryland

ASSISTANTS **M. Clark,** SUNY Upstate Medical University, Syracuse, New York
C. Copeland, University of Virginia, Charlottesville
F. Malagon, National Cancer Institute, Frederick, Maryland

The Yeast Genetics course is a modern, state-of-the-art laboratory course designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches are emphasized, including the isolation and characterization of mutants; tetrad analysis; complementation; and mitotic recombination. Molecular genetic techniques, including various types of yeast transformation, gene replacement with plasmids and polymerase chain reaction (PCR), construction and analysis of gene fusions, and generation of mutations in cloned genes, were also emphasized. Students used classical and molecular approaches to gain experience in identifying and interpreting various kinds of genetic interac-

tions, including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. They gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, green fluorescent protein (GFP)-protein fusions, and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest. This year's speakers included Joaquin Arino, Charlie Boone, Linda Breeden, Orna Cohen-Fix, Thomas Fox, Daniel Gottschling, Alan Hinnebusch, Thomas Petes, M. Mitchell Smith, George Sprague, Tim Stearns, Michael Tyers, Reed Wickner and Fred Winston.

PARTICIPANTS

Buchler, N., Ph.D., University of California, San Diego
Carneiro, T., B.S., Instituto Medicina Moelcular, Lisboa, Portugal
Coros, C., Ph.D., Wadsworth Center, Albany, New York
D'Souza, S., Ph.D., Massachusetts Institutes of Technology, Cambridge
Glick, D., Ph.D., King's College, Wilkes-Barres, Pennsylvania
Johnson, T., Ph.D., University of California, San Diego
Kamvysselis, M., M.Eng., Massachusetts Institute of Technology/Whitehead Institute, Cambridge
Kervestin, S., Ph.D., University of Massachusetts Medical School, Worcester
Krysan, D., M.D., University of Michigan, Ann Arbor
Rouse, A., B.S., Duke University, Durham, North Carolina
Sen, S., Ph.D., National Cancer Institute, Bethesda, Maryland
Sicard, D., Ph.D., University Paris XI, Gif-sur-Yvette, France
Subramaniyan, M., Ph.D., Oklahoma State University, Stillwater
Veide, J., M.Edu., Chalmers University of Technology, Göteborg, Sweden
Virtudazo, E., Ph.D., Chiba University, Japan
Wu, L., Ph.D., Harvard University, Cambridge, Massachusetts

SEMINARS

Arino, J., Universitat Autonoma de Barcelona, Spain: Phosphatases and salt tolerance.
Boone, C./Tyers, M., University of Toronto, Canada: Large-scale mapping of genetic interaction networks.
Botstein, D., Princeton University, New Jersey: Genome-scale analysis of gene expression in yeast.
Breeden, L., Fred Hutchinson Cancer Research Center, Seattle, Washington: G_1 progression in budding yeast.
Cohen-Fix, O., NIDDK, National Institutes of Health, Bethesda, Maryland: Mitotic regulation from a checkpoint perspective.
Fox, T., Cornell University, Ithaca, New York: That other genetic system: How yeast mitochondria make proteins and put them where they belong.
Gottschling, D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Hinnebush, A., NICHHD, National Institutes of Health, Bethesda, Maryland: Manifold functions of a multi-initiation factor complex in the mechanism and regulation of translation.
Petes, T., University of North Carolina, Chapel Hill: Genetic regulation of genome stability in yeast.
Smith, M., University of Virginia, Charlottesville
Sprague, G., University of Oregon, Eugene: Yeast cell-type specification and signal transduction specificity: A look back.
Stearns, T., Stanford University, California: Synthetic lethality: Then and now.
Wickner, R., National Institutes of Health, Bethesda, Maryland: Prions in yeast.
Winston, F., Harvard Medical School, Boston, Massachusetts: Analysis of factors that control transcription and chromatin structure in yeast.

Molecular Mechanisms of Human Neurological Diseases

July 23–29

INSTRUCTORS **A. Aguzzi,** University Hospital of Zurich, Switzerland
S. Gandy, Thomas Jefferson University, Philadelphia, Pennsylvania
J. Hardy, NIA, National Institutes of Health, Bethesda, Maryland

How and why do neurons dysfunction or die in specific acute or chronic human neurological disorders? What are the molecular and biochemical manifestations of specific genetic lesions in specific neurological disorders? Do different pathological dysfunctions share common mechanisms? What practical treatments are contemplated? This lecture course explored possible answers to these important questions. Recent advances in neurogenetics and in molecular and cell biology have begun to shed light on the mechanisms that underlie nervous system injury in disease states such as stroke, epilepsy, channelopathies, Alzheimer's disease, Parkinson's disease, frontotemporal dementia, amyotrophic lateral sclerosis, prion diseases, narcolepsy, and polyglutamine repeat disorders. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their area of expertise. Overview was provided and course participants did not have to be familiar with neurological diseases, although a background in basic nervous system structure and organization was extremely helpful.

The course focused principally on the specific hypotheses and approaches driving current research. Emphasis was placed on the highly dynamic interface between basic and clinical investigation including: the interdependence of clinical research and disease model development and the value of disease research in understanding the function of the normal nervous system. The course was held at the Laboratory's Banbury Conference Center.

PARTICIPANTS

Alexopoulos, H., Ph.D., University of Oxford, United Kingdom
Antal, E., M.D., University of Oslo, Norway
Ascherio, A., Ph.D., Harvard School of Public Health, Boston, Massachusetts
Atchison, K., B.S., Wyeth Research, Princeton, New Jersey
Bueler, H., Ph.D., University of Zurich, Switzerland
Carter, T., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Causevic, M., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Chappell, C., M.A., University of Oklahoma, Edmond
Gafni, J., Ph.D., Buck Institute for Research in Aging, Novato, California
Hygge Blakeman, K., Ph.D., Karolinska Institutet, Stockholm, Sweden
LaFevre-Bernt, M., Ph.D., Buck Institute for Age Research, Novato, California
Mapelli, M., Ph.D., European Institute of Oncology, Milan, Italy
McCarthy, S., B.A., Karolinska Institutet, Stockholm, Sweden
Meltzer, C., M.D., University of Pittsburgh, Pennsylvania
Messmer, K., Ph.D., University of Maryland, Baltimore
Nielsen, M., B.S., University of Copenhagen, Denmark
Orr, A., B.S., Emory University, Atlanta, Georgia
Pedrini, S., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Polymenidou, M., B.S., University Hospital of Zurich, Switzerland
Strom, A., M.A., Umea University, Umea, Sweden
Sveinbjornsdottir, S., M.D., Landspitali University Hospital, Iceland
Zuzga, D., B.A., Thomas Jefferson University, Philadelphia, Pennsylvania

SEMINARS

Aguzzi, A., University Hospital of Zurich, Switzerland: Prions.
Burke, B., Columbia University, New York: Parkinson's.
Cookson, M., NIA, National Institutes of Health, Bethesda, Maryland: Mechanisms of neurodegeneration.
Frackowiak, R., Wellcome, London, United Kingdom: Functional neuroimaging and cerebral localization.
Gandy, S., Thomas Jefferson University, Philadelphia, Pennsylvania: Alzheimer's.
Hardy, J., NIA, National Institutes of Health, Bethesda, Maryland: Neurogenetics.
Heutink, P., Erasmus University Rotterdam, The Netherlands: Novel Parkinson's genes.
Hutton, M., Mayo Clinic Jacksonville, Florida: Tauopathies.
Iadecola, C., Weill Cornell Medical Center, New York: Stroke.
MacDonald, M., Massachusetts General Hospital, Charlestown: Huntington's
McNamara, J., Duke University Medical Center, Durham, North Carolina: Epilepsy.
Murphy, D., NINDS, National Institutes of Health, Rockville, Maryland: NINDS Funding.
Nitsch, R., University of Zurich, Switzerland: Alzheimer's.
Orr, H., University of Minnesota, Minneapolis: Trinucleotide repeat diseases (SCA).
Schofield, P., The Garvan Insitute of Medical Research, Sydney, Australia: Channelopathies.
Siegel, J., Veterans Affairs Greater Los Angeles Health Care System, North Hills, California: Hypocretin/orexin physiology and pathophysiology.

Workshop on Magnetoencephalography

July 29–August 2

INSTRUCTORS **R. Llinas,** New York University School of Medicine
U. Ribary, New York University School of Medicine

ASSISTANTS **S. Jaramillo,** New York University School of Medicine
K. Moran, New York University School of Medicine
R. Ramirez, New York University School of Medicine
K. Sauve, New York University School of Medicine
J. Schulman, New York University School of Medicine

Magnetoencephalography (MEG) is a noninvasive, nonhazardous technology allowing for functional imaging of the human brain's electrophysiology at millisecond temporal resolution. Localization of electrical activity has an accuracy of approximately 2 mm. MEG simultaneously measures neuronal activity at hundreds of points in the human brain. Measurement preparation and collection times are relatively short and can be applied to spontaneous brain activity or in response to stimuli. Other brain imaging technologies including X-ray computed tomography (CT), fMRI, PET, and SPECT, generally measure indirect correlates of brain function, such as anatomy, blood flow, and oxygenation state. The core technology in MEG is based on highly sensitive superconducting quantum interference devices (SQUIDs) capable of detecting subtle fluctuations in a magnetic field associated directly with the neu-

ronal electrical activity. MEG is an emerging brain-imaging technology that has application in functional imaging in normal and pathological brain function. This five-day workshop included extended lectures and open discussion by leading practitioners in the field, as well as an extended field trip and hands-on exposure to the MEG facility at the New York University School of Medicine in New York City.

LECTURERS

Ilmoniemi, R., Nexstim Ltd., Helsinki, Finland: Signal processing and cognitive functions.
Ioannides, A., Brain Science Institute, Riken, Saitama, Japan: Magnetic field tomography (MFT) and applications.
Kronberg, E., New York University, School of Medicine: MEG analysis in time and frequency domain.
Mitra, P., Bell Laboratories, Murray Hill, New Jersey: Extraction of MEG signals and signal processing techniques.
Mosher, J., Los Alamos National Laboratory, New Mexico: Overview: Source localization techniques.
Okada, Y., University of New Mexico, Albuquerque: Interpretation of MEG signals.
Salenius, S., Helsinki University of Technology, Espoo, Finland: Sensorimotor oscillations.
Schnitzler, A., Heinrich-Heine University, Duesseldorf, Germany: Large-scale oscillatory coupling in the human brain.
Vrba, J., CTF Systems, Inc., Port Coquitlam, Canada: Signal-noise cancellation and source localization (SAM).

PARTICIPANTS

Christian, E., AstraZeneca Pharmaceuticals, Wilmington, Delaware
Best, S.D., The Neuroscience Center, Deerfield, Illinois
Hironaga, N., Brain Science Institute, Riken, Saitama, Japan
Nurminen, J., BioMag Laboratory, Helsinki, Finland
Poghosyan, V., Brain Science Institute, Riken, Saitama, Japan
Polyakov, A., Weill Cornell Medical Center, New York
Zaman, S., Guys and Maudsley Hospital, London, United Kingdom

Cellular Biology of Addiction

August 4–11

INSTRUCTORS **B. Madras,** Harvard Medical School, Southborough, Massachusetts
M. Von Zastrow, University of California, San Francisco

ASSISTANT **G. Miller,** Harvard Medical School, Southborough, Massachusetts

Drug addition is the most costly neuropsychiatric disorder faced by our nation. The primary objective of this workshop was to provide an intense dialogue of the fundamentals, state-of-the-art advances, and major gaps in the cellular and molecular biology of drug addiction. Targeted to new or experienced investigators, the workshop combined formal presentations and informal discussions to convey the merits and excitement of cellular and molecular approaches to drug addiction research.

With the advent of genomics and proteomics, an extraordinary opportunity now exists to develop comprehensive models of neuroadaptative processes fundamental to addiction, craving, and relapse to drug use and to brain function, in general. During the workshop, a range of disciplines and topics were represented, including noninvasive brain imaging to identify drug targets and adaptive processes, neuroadaptative processes at the molecular and celllular levels, neural networks and their modulation, the relevance of genotype to susceptibility and drug response, tolerance and adaptation at the cellular level, and neuroinformatics.

This workshop provided an integrated view of current and novel research on neuroadaptive responses to addition, fostered discussion on collaboration and integration, and provided critical information needed to construct a model of addiction as a disease and novel molecular targets for biological treatments. Beyond the plane of scientific endeavor, the information is vital for formulating public policy and for enlightening the public to the neurobiological consequences of drug use and addiction. The workshop was designed to generate interest in this approach, open conduits for collaborations, and provide novel routes for investigating the neurobiology of addiction.

PARTICIPANTS

Achat, C., B.S., University of Miami School of Medicine, Florida
Agoston, D., Ph.D., Uniformed Services University of the Health Sciences, Bethesda, Maryland
Cancela, L., Ph.D., Universidad Nacional de Cordoba, Argentina
Chiang, L., Ph.D., Purdue Pharma L.P., Cranbury, New Jersey
Du, C., Ph.D., Brookhaven National Laboratory, Upton, New York
Febo, M., Ph.D., University of Massachusetts Medical School, Worcester
Gerdeman, G., Ph.D., The University of Arizona Health Sciences Center, Tucson
Ghitza, U., B.A., Rutgers University, Piscataway, New Jersey
Glaser, S., B.S., Brookhaven National Laboratory, Upton, New York
Grice, D., M.D., University of Pennsylvania, Philadelphia
Guan, Z., M.D., Columbia University, New York
Kumra, S, M.D., Albert Einstein College of Medicine, Bronx, New York
Marini, A., M.D., Uniformed Services University of the Health Sciences, Bethesda, Maryland
Martell, B., M.D., Yale University School of Medicine, W. Haven, Connecticut
Milby, J., Ph.D., University of Alabama, Birmingham
Miller, C., M.S., University of California, Irvine
Morrow, E., Ph.D., Massachusetts General Hospital, Boston
Ryan, X., Ph.D., The Rockefeller University, New York
Schiffer, W., B.A., Stony Brook University/Brookhaven National Laboratory, Upton, New York
Stridh, M., M.S., Karolinska Institutet, Stockholm, Sweden
Thomsen, M., M.S., McLean Hospital/Harvard Medical School, Belmont, Massachusetts
Torres-Reveron, A., B.S., SUNY Downstate Medical Center, Brooklyn, New York
Twining, R., B.A., Pennsylvania State College of Medicine, Hershey
Verrico, C., Ph.D., Harvard Medical School/NEPRC, Southborough, Massachusetts
Yao, L., Ph.D., University of California, San Francisco

SEMINARS

Augustine, G., Duke University Medical Center, Durham, North Carolina: Synaptic transmission and drug addition.
Breiter, H., Massachusetts General Hospital, Boston
Brown, E., Massachusetts General Hospital, Boston
Edwards, R., University of California, San Francisco
Gasic, G., Massachusetts General Hospital, Boston
George, S., University of Toronto, Canada
Goldman, D., NIAAA/LNG, Rockville, Maryland
Kosofsky, B., Massachusetts General Hospital, Boston: Developmental aspects of addiction.
Kreek, M.J., The Rockefeller University, New York
Lake, G., Institute for Systems Biology, Seattle, Washington
Madras, B., Harvard Medical School, Southborough, Massachusetts
Malenka, R., Stanford University School of Medicine, California: Role of synaptic plasticity in addiction.
Nestler, E., University of Texas Southwestern, Dallas
Pollack, J., NIDA, Bethesda, Maryland
Schwartz, J., Columbia University, New York
Uhl, G., NIDA, Bethesda, Maryland
von Zastrow, M., University of California, San Francisco: Effects of opiate drugs on the cellular regulation of opioid receptors: Mechanisms and physiological consequences.
Worley, P., Johns Hopkins University School of Medicine, Baltimore, Maryland
Zhou, R., Rutgers University, Piscataway, New Jersey: Ontogeny of the midbrain dopaminergic pathways.

Bioinformatics: Writing Software for Genome Research

October 15–28

INSTRUCTORS **S. Lewis,** Hospital for Sick Children Research Institute, Toronto, Ontario, Canada
G. Hartzell, University of California, Berkeley
L. Stein, Cold Spring Harbor Laboratory

ASSISTANTS

S. Cain, Cold Spring Harbor Laboratory
A. Day, University of California, Los Angeles
R. Halgren, Michigan State University, E. Lansing
J. Kaminker, University of California, Berkeley
J. Kissinger, University of Pennsylvania, Philadelphia
A. Mackey, University of Virginia, Charlottesville
D. Nix, University of California, Berkeley
S. Prochnik, University of California, Berkeley
S. Robb, University of Utah, Salt Lake City
C. Smith, University of California, Berkeley
J. Stajich, Duke University, Durham, North Carolina
L. Teytelman, Cold Spring Harbor Laboratory
J. Tupy, University of California, Berkeley
C. Wiel, University of California, Berkeley

The desktop computer is rapidly becoming an indispensable tool in the biologist's toolchest. The success of the Human Genome Project created an explosion of information: billions of bits of biological information stashed electronically in databases around the globe just waiting for the right key to unlock them. New technologies such as DNA microarrays and high-throughput genotyping are creating an information overload that the traditional laboratory notebook cannot handle. To exploit the information

revolution in biology, biologists moved beyond canned Web interfaces and Excel spreadsheets. They took charge of the data by creating their own software to fetch, manage, and integrate it. The goal of this course was to provide biologists with the tools needed to deal with this changing landscape. Designed for students and researchers with little prior knowledge of programming, this two-week course taught the fundamentals of the Unix operating system, Perl scripting, dynamic Web page development with the CGI protocol, and database design. The course combined formal lectures with hands-on experience in which students worked to solve a series of problem sets drawn from common scenarios in biological data acquisition, integration, and laboratory workflow management. For their final projects, students posed problems using their own data and worked with each other and the faculty to solve them.

PARTICIPANTS

Adachi, H., Ph.D., Kyushu University, Japan
Aerts, J., M.S., Wageningen University, The Netherlands
Ambroggio, X., B.S., California Institute of Technology, Pasadena
Arteaga-Vazquez, B.S., CINVESTAV-IPN, Guanajuato, Mexico
Averof, M., Ph.D., IMBB-FORTH, Greece
Boardman, A., B.S., South African National Bioinformatics Institute, Bellville, South Africa
Chamnapunt, J., Ph.D., Kasetsart University, Thailand
Childers, C., B.S., Texas A&M University, College Station
Dimova, D., Ph.D., Harvard University, Charlestown, Massachusetts
Fiedler, T., Ph.D., University of Miami Rosenstiel School, Florida
Fox, J., Ph.D., UBC Bioinformatics Centre, Vancouver, Canada
Hayden, K., B.S., Case Western Reserve University, Cleveland, Ohio
Hild, M., Ph.D., Zentrum für Molekulare Biologie, University of Heidelberg, Germany
Hildebrandt, J., Ph.D., Medical University of South Carolina, Charleston
Holmes, J., M.P.A., Oklahoma Medical Research Foundation, Oklahoma City
Hughes, J., Ph.D., Whitehead Institute, Cambridge, Massachusetts
McMahon, W., B.S., Texas Technical University Health Sciences Center, Lubbock
Moore, M., M.S., University of Utah, Salt Lake City
Petri, A., M.S., Hagedorn Research Institute, Gentofte, Denmark
Reyes-Valdes, M., Ph.D., Universidad Autonoma Agraria Antonio Narro, Coah, Mexico
Telford, M., Ph.D., University of Cambridge, United Kingdom
Tsipouri, V., M.S., National Human Genome Research Institute, Bethesda, Maryland
Wormsley, S., Ph.D., Yale University, New Haven, Connecticut
Wu, J., M.S., Human Genome Sequencing Center, Houston, Texas
Xie, Q., Ph.D., GlaxoSmithKline, King of Prussia, Pennsylvania

SEMINARS

Hide, W., University Western Cape, SANBI, Bellville, South Africa: EST clustering.
Jamison, C., George Mason University, Manassas, Virginia: Using genomic databases effectively.
Marth, G., NCBI, National Institutes of Health, Bethesda, Maryland: Sequence variation analysis.
Ostell, J., National Institutes of Health, Bethesda, Maryland: The NCBI tools.
Pearson, W., University of Virginia, Charlottesville: Sequence similarity analysis.
Peitzsch, R., Pfizer Global R&D, Groton, Connecticut: Modeling biological data in relational databases.
Troyanskay, O., Stanford University, California: Microarray analysis.
Yandell, M., University of California, Berkeley: The BLAST algorithm.

X-ray Methods in Structural Biology

October 15–30

INSTRUCTORS **W. Furey,** V.A. Medical Center, Pittsburgh, Pennsylvania
G. Gilliland, Center for Advanced Research in Biotechnology, Gaithersburg, Maryland
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku/MSC, Inc., The Woodlands, Texas

ASSISTANTS **B. Narayanan,** Center for Advanced Research in Biotechnology, Gaithersburg, Maryland

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensified laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics covered included basic diffraction theory, crystallization (proteins, nucleic acids, and

complexes), crystal characterization, X-ray sources and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction, molecular replacement, solvent flattening, noncrystallographic symmetry averaging, electron density interpretation, structure refinement, molecular graphics, and coordinate deposition. Participants learned through extensive hands-on experiments. They crystallized and determined a protein structure, in parallel with lectures on the theory and informal discussions behind the techniques. Applicants were familiar with the creation and editing of simple text files on UNIX workstations using a screen-based editor (either vi or emacs).

PARTICIPANTS

Bickford, L., B.S., Memorial Sloan-Kettering Cancer Center, New York
Chiu, C., B.S., University of British Columbia, Vancouver, Canada
De Marco, V., Ph.D., Netherlands Cancer Institute, Amsterdam, The Netherlands
Deng, X., M.S., University of Nebraska Medical Center, Omaha
First, E., Ph.D., Louisiana State University Health Sciences Center, Shreveport
Garces, R., B.S., University of Toronto, Canada
Hillson, N., B.A., Harvard Medical School, Boston, Massachusetts
Jenni, S., B.S., Swiss Federal Institute of Technology, Zurich, Switzerland
Larsen, S., Ph.D., University of Illinois, Chicago
Lougheed, J., Ph.D., Exelixis, Inc., San Francisco, California
Newitt, J., Ph.D., Bristol-Myers Squibb Company, Princeton, New Jersey
Richter-Addo, G., Ph.D., University of Oklahoma, Norman
Roberts, J., B.S., University of Colorado, Boulder
Strobel, S., Ph.D., Yale University, New Haven, Connecticut
Van Train, K., B.S., Thomas Jefferson University, Philadelphia, Pennsylvania
Von Koenig, K., B.S., Max-Planck-Institute for Molecular Physiology, Dortmund, Germany

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, Berkeley, California: Introduction to CNS. CNS macromolecular refinement.
Dauter, Z., Brookhaven National Laboratory: Practical considerations for synchrotron data collection and for SAD and MAD phasing.
Furey, W., V.A. Medical Center, Pittsburgh, Pennsylvania: Isomorphous replacement and anomalous scattering I. Isomorphous replacement and anomalous scattering II. Patterson group therapy. Phase improvement by solvent flattening/negative density transaction. Noncrystallographic symmetry averaging. Crystallographic symmetry operations and their application.
Gilliland, G., National Institute of Standards & Technology, Gaithersburgh, Maryland: The BMCD: Crystallization data strategies. Molecular replacement.
Hendrickson, W., Columbia University, New York: MAD phasing: Theory and practice.
Hung, L., Brookhaven National Laboratory, Upton, New York: SOLVE; RESOLVE.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Two intermediates in the assembly of the papillomavirus replication initiation complex. Structure presentation.
Kjeldgaard, M., Aarhus University, Aarhus, Denmark: Electron density fitting A to O.
Kleywegt, G., University of Uppsala, Sweden: Just because it's in nature, doesn't mean it's true...(macromolecular structure validation).
McPherson, A., University of California, Irvine: Crystallization of macromolecules I and II. Symmetry, pediodicity, unit cells, space groups, miller planes, and lattices. The structure factor and Friedel's law. Fundamental diffraction relationships and Bragg's law. Diffraction patterns, reciprocal space, and Ewald's sphere. Fourier transforms and the electron density equation. Pattern techniques. Crystallization review and optimization. Heavy atoms and anomalous scatterers.
Perrakis, A., Nederland Kanker Institute, Amsterdam, The Netherlands: ARP/wARP: The underlying concepts and algorithms for automated procedures for phase improvement, extension, and model building. ARP/wARP: Implementation and examples of applications.
Pflugrath, J., Rigaku/MSC, Inc., The Woodlands, Texas: Data collection: Design and setup I and II. Cryocrystallography. Scaling and merging synchrotron data. Application of anomalous scattering from sulfur atoms.
Richardson, D., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using all atom contacts.
Sweet, R., Brookhaven National Laboratory, Upton, New York: Fundamentals of crystallography. X-ray sources and optics.
Tronrud, D., University of Oregon, Eugene: Macromolecular refinement I and II.
Westbrook, J., Rutgers University, Piscataway, New Jersey: The protein data bank.
Whitby, F., University of Utah School of Medicine, Salt Lake City: Crystal structures of uroporphyrinogen decarboxylase enzyme-product complexes.
Xu, R.-M., Cold Spring Harbor Laboratory: Structural basis of histone methylation.

Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 18–31

INSTRUCTORS **A. Dernburg,** Lawrence Berkeley National Laboratory, Berkeley, California
J. Murray, University of Pennsylvania School of Medicine, Philadelphia
J. Swedlow, University of Dundee, United Kingdom

ASSISTANTS **K. Hu,** Scripps Research Institute, La Jolla, California
W. Moore, University of Dundee, United Kingdom
J. Peng, University of California, Berkeley
S. Suravajjala, University of Pennsylvania School of Medicine, Philadelphia

This course focused on specialized techniques in microscopy, in situ hybridization, immunocytochemistry, and live cell imaging related to localizing DNA, RNA, and proteins in fixed cells, as well as protein and RNA dynamics in living cells. The course emphasized the use of the latest equipment and tech-

niques in fluorescence microscopy, including confocal laser-scanning microscopy, deconvolution methods, digital image processing, and time-lapse imaging of living specimens. The course was designed to present students with state-of-the-art technology and scientific expertise in the use of light microscopy to address basic questions in cellular and molecular biology. The course was designed for the molecular biologist who is in need of microscopic approaches and for the cell biologist who is not familiar with the practical application of the advanced techniques presented in the course. Among the methods presented were the preparation of tagged nucleic acid probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, comparative genomic hybridization, cellular localization of RNA, localization of nucleic acids and proteins in the same cells, use of a variety of reporter molecules and nonantibody fluorescent tags, indirect antibody labeling, detection of multiple proteins in a single cell, and the use of GFP (green fluorescent protein) variants to study protein expression, localization, and dynamics. In each method, several experimental protocols were presented, allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring their own nucleic acid, protein, or antibody probes to the course, which were used in addition to those provided by the instructors. The laboratory exercises were supplemented with lectures given by invited distinguished scientists, who presented up-to-the-minute reports on current methods and research using the techniques being presented.

PARTICIPANTS

Appolioni, I., Laureate, University of Pisa, Italy
Benko, S., M.S., University of Debrecen, Hungary
Chakravarty, S., B.S., Albert Einstein College of Medicine, Bronx, New York
D'Agostino, A., Lic, University of Buenos Aires, Argentina
Gould, H., Ph.D., King's College London, United Kingdom
Hasholt, L., M.S., University of Copenhagen, Denmark
Kiesler, E., M.S., Stockholm University, Sweden
Le Baccon, P., Ph.D., Curie Institute, Paris, France
Longart, M., Ph.D., NICHD, National Institutes of Health, Bethesda, Maryland
Minter, L., Ph.D., University of Massachusetts, Amherst
Petrie, R., M.S., McGill University, Montreal, Quebec, Canada
Riazuddin, S., Ph.D., NIDCD, National Institutes of Health, Rockville, Maryland
Smart, S., Ph.D., University of California, San Francisco
Stansell, E., B.A., University of Alabama, Birmingham
Volpe, T., Ph.D., Cold Spring Harbor Laboratory
Williams, V., B.S., University of California, Riverside

SEMINARS

Belmont, A., University of Illinois, Urbana: Using GFP marks to study nuclear dynamics.
Day, R., University of Virginia, Charlottesville: Seeing colors: Applications and limitations of the fluorescent proteins.
Dernburg, A., Lawrence Berkeley National Laboratory, Berkeley, California: Basics of FISH.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Basic introduction to light and fluorescence microscopy. Immunocytochemistry.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Principles of confocal microscopy and deconvolution techniques.
Ried, T., NCI, National Institutes of Health, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Spector, D., Cold Spring Harbor Laboratory: Localization of gene expression by FISH and in living cells.
Swedlow, J., University of Dundee, United Kingdom: Live-cell imaging. Principles of confocal microscopy and deconvolution techniques.
Tran, P., University of Pennsylvania, Philadelphia: Cameras and digital imaging fundamentals.

Phage Display of Combinatorial Antibody Libraries

November 4–17

INSTRUCTORS **C. Barbas,** The Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, University of California, San Diego

ASSISTANTS **C. Goodyear,** University of California, San Diego
K. Noren, New England Biolabs, Beverly, Massachusetts
C. Tuckey, New England Biolabs, Beverly, Massachusetts

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This intensive laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources, as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. coli* were also covered. Epitopes were selected from peptide libraries and characterized.

The lecture series was presented by a number of invited speakers, which emphasized polymerase chain reaction (PCR) of immunoglobulin genes, the biology of filamentous phage and the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, directed protein evolution, retroviral and cell display libraries, the immunobiology of the antibody response, and recent results on the use of antibodies in therapy. The theory and practical implications for selection from phage displayed libraries of random peptides, cDNA products, and semisynthetic proteins were also explored.

PARTICIPANTS

Agindotan, B., Ph.D., University of Idaho, Moscow
Andersen, J., Ph.D., Cold Spring Harbor Laboratory
Basquin, D., B.S. University of Geneva, Switzerland
Chen, C., Ph.D., Commonwealth Serum Laboratories, Ltd., Parkville, Australia
Clancy, R., Ph.D., Hospital for Joint Diseases, New York
Kjaergaard, K., Ph.D., Hagedorn Research Institute, Bagsvaerd, Denmark
Larman, B., B.S., University of Southern Maine, Portland
Lee, D., M.B.A., Cedrus Technologies, Inc., Seattle, Washington
LeMosy, E., Ph.D., Medical Collge of Georgia, Augusta
O'Nuallain, B., Ph.D., University of Tennessee Medical Center, Knoxville
Persson, H., M.S., Lund University, Lund, Sweden
Popov, B., Ph.D., Northwestern University, Chicago, Illinois
Song, Z., Ph.D., National University of Singapore, China
Su, S., Ph.D., University of California, Los Angeles
Yang, S., Ph.D., Kimberly-Clark Corporation, Roswell, Georgia

SEMINARS

Austin, D., Yale University, New Haven, Connecticut: T7 phage display and interaction cloning.
Barbas, C., The Scripps Research Institute, La Jolla, California: Software and hardware for genomes: Polydactyl zinc finger proteins and the control of endogenous genes.
Lowman, H., Genetech, Inc., S. San Francisco, California: SAR of peptides using phage.
Marks, K., Stanford University School of Medicine, California: Retroviral libraries.
Noren, C., New England Biolabs, Beverly, Massachusetts: Phage peptide libraries: The PhD for peptides.
Pasqualini, R., University of Texas, Houston: In vivo panning.
Sidho, S., Genetech, Inc., S. San Francisco, California: Use of antibody-phage libraries to develop antibodies as reagents and potential therapeutics.
Siegel, D., University of Pennsylvania Medical Center, Philadelphia: Cell surface selection of combinatorial Fab libraries.
Silverman, G., University of California, San Diego: Repertoire cloning of SLE autoantibodies.
Webster, R., Duke University, Durham, North Carolina: The biology of filamentous phage.
Wilson, I., The Scripps Research Institute, La Jolla, California: Structural biology of the immune system.

Proteomics

November 4–17

INSTRUCTORS **P. Andrews,** University of Michigan Medical School, Ann Arbor
J. La Baer, Harvard Medical School, Boston, Massachusetts
B. Phinney, Michigan State University, E. Lansing

ASSISTANTS **E. Hainsworth,** Harvard Medical School, Boston, Massachusetts
N. Ramachandran, Harvard Institute of Proteomics, Cambridge, Massachusetts
D. Veine, University of Michigan, Ann Arbor
D. Whitten, Michigan State University, E. Lansing

This intensified laboratory and lecture course focused on two major themes in proteomics. In the profiling section of the course, students learned methodologies of protein preparation from diverse samples, sample analysis by two-dimensional gel electrophoresis, high-sensitivity mass spectrometric analysis of proteins utilizing both peptide mass mapping and tandem mass spectrometry, and the application of bioinformatics tools to identify proteins and posttranslational modifications and assess their relative abundance. In the functional proteomics section of the course, students learned the use of recombinational cloning to move many genes simultaneously to different expression vectors, and how to perform high-throughput expression, purification, and characterization of proteins, and how to produce and analyze protein microarrays. Students learned approaches to identify protein interactions

utilizing affinity isolation techniques coupled with mass spectrometric protein identification. The overall aim of the course was to provide students with the fundamental knowledge and hands-on experience necessary to be able to perform and analyze proteomics experiments, and to learn to identify new opportunities in applying proteomics approaches to their own research.

PARTICIPANTS

Arrigoni, R., Laureate, Albert Einstein College of Medicine, Bronx, New York
Bokov, A., B.S., University of Texas Health Science Center, San Antonio
Bujold, E., M.D., Wayne State University, Detroit, Michigan
Choi, S., M.D., Stony Brook University, New York
Fahrni, M., Ph.D., Center of Disease Control and Prevention, Atlanta, Georgia
Gazula, V., Ph.D., Yale University, New Haven, Connecticut
Hainsworth, E., M.S. Harvard Medical School, Boston, Massachusetts
Julin, K., M.S., Norwegian Institute of Fisheries & Aquaculture Research, Tromso, Norway
Levert, K., Ph.D., Centers for Diseases Control and Prevention, Atlanta, Geogia
Montor, W., B.S., University of Sao Paulo, Brazil
Morris, D., M.D., University of California, San Francisco
Ofek, P., M.S., Tel Aviv University, Tel Aviv, Israel
Sanders, P., B.S., University of Cambridge, United Kingdom
Stevens-Truss, R., Ph.D., Kalamazoo College, Michigan
Thierse, H., Ph.D., Max-Planck-Institute for Immunobiology, Freiburg, Germany
Thomassen, E., M.S., University of Tormso, Norway

SEMINARS

Chait, B., The Rockefeller University, New York: Phosphoprotein analysis in proteomics.
Chaurand, P., Vanderbilt University School of Medicine, Nashville, Tennessee: Imaging by mass spectrometry.
Gerber, S., Harvard Medical School, Boston, Massachusetts: Quantitative methods in mass spectrometry.
MacBeath, G., Harvard University, Cambridge, Massachusetts: Protein microarrays.
Snyder, M., Yale University, New Haven, Connecticut: Protein microarrays.
Tempst, P., Memorial Sloan-Kettering Cancer Center, New York: Biomarker discovery in mass spectrometry.
Washburn, M., Stowers Institute for Medical Research, Kansas City, Missouri: Ion-trap mass spectrometry.

Computational Genomics

November 5–11

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
R. Smith, GlaxoSmithKline, King of Prussia, Pennsylvania

ASSISTANTS **I. Ovcharenko,** Lawrence Livermore National Laboratory, Livermore, California
B. Cantarel, University of Virginia, Charlottesville

Beyond BLAST and FASTA—This course presented a comprehensive overview of the theory and practice of computational methods for gene identification and characterization from DNA sequence data. The course focused on approaches for extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, and multiple sequence alignment. Additional topics included gene recognition (exon/intron prediction), identifying signals in unaligned sequences, and integration of genetic and sequence information in biological databases. The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course made extensive use of local WWW pages to present problem sets and the computing tools to solve them. Students used Windows

and Mac workstations attached to a UNIX server; participants had to be comfortable using the UNIX operating system and a UNIX text editor. The course was designed for biologists seeking advanced training in biological sequence analysis, computational biology core resource directors and staff, and scientists in other disciplines, such as computer science, who wish to survey current research problems in biological sequence analysis.

PARTICIPANTS

Chan, A., Ph.D., The Institute for Genomic Reseach, Germantown, Maryland
De Bondt, A., Ph.D., Johnson & Johnson, Beerse, Belgium
Fiedler, T., Ph.D., University of Miami Rosenstiel School Marine & Atmosphere Science, Florida
Gallagher, T., B.S., Darmouth Medical School, Hanover, New Hampshire
Good-Avila, S., Ph.D., Acadia University, Nova Scotia, Canada
Hammerli, A., B.S., Yale University, New Haven, Connecticut
Hansen, N., Ph.D., NIH Intramural Sequencing Center, Gaithersburg, Maryland
Henthorn, P., Ph.D., University of Pennsylvania School of Veterinary Medicine, Philadelphia
Hildebrandt, J., Ph.D., Medical University of South Carolina, Charleston
Karro, J., Ph.D., Yale University, New Haven, Connecticut
Kim, H., M.S., Baylor College of Medicine, Houston, Texas
Larsson, P., M.S., Uppsala University, Sweden
Martinez de la Vega, O., Ph.D., CINVESTAV, Guanajuato, Mexico
Matasci, N., B.S., Max-Planck-Institute for Evolutionary Anthropology, 1 Leipzig, Germany
Mattingly, C., Ph.D., Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine
Moy, C., B.S., University of Pennsylvania, Philadelphia
Nikolskaya, A., Ph.D., Georgetown University, Washington, D.C.
Ramamurthy, L., Ph.D., GlaxoSmithKline, Research Triangle Park, North Carolina
Smits, B., M.Sc., Hubrecht Laboratory, Utrecht, The Netherlands
Somerville, C., Ph.D., Marshall University, Huntington, Wyoming
Tang, K., Ph.D., National Center for Infectious Disease/Centers for Disease Control & Prevention, Atlanta, Georgia
Trutschl, M., Sc.D., Louisiana State University and Louisiana State University Health Sciences Center, Shreveport
Viswanathan, R., Ph.D., Beloit College, Wisconsin
Wienholds, E., M.S., Hubrecht Laboratory, Utrecht, The Netherlands

SEMINARS

Altschul, S., National Library of Medicine, Bethesda, Maryland: Statistics of sequence similarity scores. Iterated protein database searches with PSI-BLAST.
Brent, M., Washington University, St. Louis, Missouri: Eukaryotic gene finding; Comparative methods for gene finding.
Cooper, P., National Center for Biotechnology Information, Bethesda, Maryland: NCBI Resources for bioinformatics and computational biology. NCBI genome resources.
Mackey, A., University of Virginia, Charlottesville: Displaying/examining genomes.
Pearson, W., University of Virginia, Charlottesville: Protein evolution and sequence similarity searching. Hidden Markov models and protein profiles. Alignment algorithms—large-scale alignment. Identifying motifs.
Smith, R., GlaxoSmithKline, King of Prussia, Pennsylvania: Approaches to multiple sequence alignment. Multiple alignment resources.
Stubbs, L., Lawrence Livermore National Laboratory, Livermore, California: Algorithms for genome comparison. Genome comparison biology.
Yandell, M., University of California, Berkeley: Chado, ontologies for annotation. Genome annotation.

The Genome Access Course

February 4–5, April 8–9, June 10–11, September 30–October 1

TRAINERS **J. Gergel, M. Katari, L. Palmer, and E. Thomas**, Cold Spring Harbor Laboratory
U. Hilgert, Dolan DNA Learning Center

Initiated in 2002, this course is an intensive two-day introduction to bioinformatics that was held multiple times in 2003 and trained almost 110 participants in total. The core of the course was designed to cover the manipulation and analysis of sequence information. The course was broken into modules designed to give a broad overview of a given topic, with ample time for examples chosen by the instructors. Each module included three parts, consisting of a discussion of theory and methods, coverage of software and Web resources, and use of selected tools with examples (including those supplied by the students). The modular design allowed the instructors to tailor the presentation to the interests of the students. Modules included Electronic Sequence Information; Pairwise Sequence Comparisons; Multiple Sequence Alignments; Gene Prediction; Genome Analysis; Sequence Variation; Protein Classification and Structural Analysis; Proteomics; and Phylogenetic Analysis. The course was held at the Laboratory's Genome Research Center at Woodbury. Each student was provided with a PC laptop with wireless modem for the duration of the course. Students were encouraged to supply problem sets and sequences of interest to the trainers for possible incorporation as examples in the modules. Materials were made available on the Web and students continued to ask questions of the trainers as they applied what they had learned in their individual endeavors.

Genome Access Course, February 2003

February 4–5

Akassoglou, K., Ph.D., New York University Medical Center/ Skirball, New York
Beutler, A., Ph.D., Mount Sinai School of Medicine, New York
Caporale, L., Ph.D., Independent researcher, New York
Carlson, K., B.S., Concord, Massachusetts
Chen, J., Ph.D., Boston University School of Medicine, Massachusetts
Deschamps, D.M., B.S., Hopkinton, Massachusetts
Fryer, R., M.D., The Rockefeller University and Columbia University, New York
Li, Z., Weill Medical College, Cornell University, New York
Lu, L., Ph.D., Dana-Farber Cancer Institute Harvard Medical School, Boston, Massachusetts

Malisetty, V., Ph.D., Institute for Cancer Prevention, Valhalla, New York
McGarry, T., Ph.D., Northwestern University Medical School, Chicago, Illinois
Patlolla, J. M., M.D., American Health Foundation, Valhalla, New York
Richler, E., B.S., Mount Sinai School of Medicine, New York
Robins, H., Ph.D., Institute for Advanced Study, Princeton, New Jersey
Rodriguez-Contreras, A., Ph.D., University of California, Davis
Silva, J., B.S., Karolinska Institutet, Huddinge, Sweden
Singh, M., Ph.D., Vanderbilt University, Nashville, Tennessee
Su, H., Ph.D., Regeneron Phamaceuticals, Tarrytown, New York
Telesnitsky, A., Ph.D., University of Michigan, Ann Arbor
Vitarius, J., Ph.D., The Rockefeller University, New York
Walter, K., M.D., University of Pittsburgh, Pennsylvania
Wang, B., Ph.D., Weill Medical College, New York
Watson, D., Ph.D., Southern Illinois University, Carbondale
Wong, J., B.S., Brown University, Providence, Rhode Island
Xue, Y., B.S., Regeneron Phamaceuticals, Tarrytown, New York
Zhang, P., B.S., Harvard University School of Public Health, Boston, Massachusetts

Genome Access Course, April 2003

April 8–9

DuttaGupta, R., B.S., University of Medicine and Dentistry, Piscataway, New Jersey
Foster, L., Ph.D., Trinity College, Hartford, Connecticut
Gawel, C., B.S., North Shore–Long Island Jewish Research Institute, Manhassett, New York
Heath, M., M.D., Columbia University, New York
Huang, Y., B.S., University of Massachusetts Medical School, Worcester
Kuo, P., Ph.D., Duke University, Durham, North Carolina
Li, X., Ph.D., Senomyx, Inc., La Jolla, California
Li, Y., Ph.D., HHMI and Skirball Institute, New York University School of Medicine, New York
Lin, J., B.S., Cold Spring Harbor Laboratory
Maillard, I., B.S., University of Pennsylvania, Philadelphia
May, B., Ph.D., Cold Spring Harbor Laboratory
Ramirez, N., B.S., Ohio State University, Wooster
Santhanam, A., B.S., University of Medicine and Dentistry of New Jersey, Newark
Singh, S., Ph.D., Weill Medical College, New York
Volk, E., B.S., Ohio State University/OARDC, Wooster
Wang, J., Ph.D., Weill Medical College of Cornell University, New York
Yu, L., Ph.D., Boston University School of Medicine, Massachusetts

June 10–11

Aneja, K., B.S., St. John's University, Fresh Meadows, New York
Aplenc, R., Ph.D., CHOP and University of Pennsylvania, Philadelphia
Avraham, S., B.S., Cold Spring Harbor Laboratory
Behe, M., Ph.D., Lehigh University, Bethlehem, Pennsylvania
Citek, R., Ph.D., Orion Genomics, Saint Louis, Missouri
Dauer, W., Ph.D., Columbia University, New York
Ferguson, G., Ph.D., Massachusetts Institute of Technology, Cambridge
Godoy, V., B.S., Massachusetts Institute of Technology, Cambridge

Genome Access Course, June 2003

Goodchild, R., Ph.D., Columbia University, New York
Grant, S., Ph.D., BioVentures, Inc., Murfreesboro, Tennessee
Higuchi, D., B.S., HHMI/Washington University School of Medicine, St. Louis, Missouri
Lawrence, W., B.S., Amgen Corporation, Seattle, Washington
Lazebnik, Y., Ph.D., Cold Spring Harbor Laboratory
Leon, J., Ph.D., Universidad de Cantabria, Santander, Spain
Li, Z., Ph.D., Celera Genomics, Rockville, Maryland
Liu, Y., Ph.D., Cold Spring Harbor Laboratory
Mahnke, L., Ph.D., Washington University School of Medicine, Saint Louis, Missouri
O'Brien, M., B.S., Cold Spring Harbor Labortory
Rabinowicz, P., Ph.D., Cold Spring Harbor Laboratory
Scott, Ph.D., C., Cold Spring Harbor Laboratory
Smith, D., B.S., University of California, Los Angeles
von Roeschlaub R., B.S., Cold Spring Harbor Laboratory
Ward, B., B.S., Johns Hopkins University, Baltimore, Maryland
Womble, K., B.S., BioVentures, Inc., Murfreesboro, Tennessee
Yang, X., Ph.D., National Institutes of Health, Bethesda, Maryland

September 30–October 1

Adkins, D., Ph.D., College of Charleston, South Carolina
Agindotan, B., Ph.D., University of Idaho, Moscow
Bernat, J., B.S., University of Michigan, Ann Arbor
Byrd, B., B.S., Tulane University, New Orleans, Louisiana
Choi, Y., Ph.D., Amherst College, Massachusetts
Chu, I., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Chu, T., B.S., Mount Sinai School of Medicine, New York

Genome Access Course, September/October 2003

Colborn, J., B.S., Tulane University, New Orleans, Louisiana
Connett, M., Ph.D., ArborGen LLC, Summerville, South Carolina
Gloesmann, M., Ph.D., Harvard Medical School, Boston, Massachusetts
Gopinathrao, G., Ph.D., Cold Spring Harbor Laboratory
Hill, S., Ph.D., Environment Canada, Burlington, Canada
Javaherian, A., Ph.D., Cold Spring Harbor Laboratory
Jensen-Pergakes, K., B.S., Celgene, San Diego, California
Khuong, N., Ph.D., California Department of Health Services, Richmond, California
Kim, S., Ph.D., Amherst College, Massachusetts
Kim, J., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Kontoyianni, M., Ph.D., Johnson & Johnson Pharmaceutical Research & Development, Plymouth Meeting, Pennsylvania
Kunec, D., B.S. Mississippi State University, Starkville
Leem, S., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Melnick, A., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Millar, K., B.S., Environment Canada, Burlington, Ontario, Canada
Nader, G., Ph.D., Children's National Medical Center, Washington, D.C.
Peram, L., M.D., Eric Williams Medical Sciences Complex, Trinidad, Champs Fleurs, Trinidad and Tobago
Pratt, L., Ph.D., Rockland Immunochemicals, Boyertown, Pennsylvania
Walton, M., B.S., Cleveland State University, Ohio

The Laboratory would like to acknowledge the generosity of the following companies who loaned equipment and reagents to the various courses:

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SEMINARS

Invited Speaker Program

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their findings on a weekly basis. These seminars keep the CSHL staff current on the latest developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

January

Dr. Charles Sawyers, University of California, Los Angeles, Hematology–Oncology. Kinase inhibitors in cancer. (Host: Scott Lowe)
Dr. Helen Saibil, Department of Crystallography, Birkbeck College, London. GroEL-GroES chaperonins: A protein folding machine. (Host: Leemor Joshua-Tor)

February

Dr. Constance L. Cepko, Howard Hughes Medical Institute, Harvard Medical School. Genomics approaches to photoreceptor development and disease. (Host: Josh Huang)
Dr. Michael N. Shadlen, Howard Hughes Medical Institute, University of Washington. Banburismus and brain: A neural mechanism for making decisions. (Host: Tony Zador)
Dr. Robert Eisenman, Fred Hutchinson Cancer Research Center. The *Myc* oncogene: Walking through the garden of forking paths. (Host: Bill Tansey)

March

Dr. John Doebley, University of Wisconsin, Madison. The evolution of plant form: An example from maize. (Host: David Jackson)
Dr. Harold Varmus, Memorial Sloan-Kettering Cancer Center. Using mouse models to learn how cancers arise and survive. (Host: Bruce Stillman)
Dr. Joan W. Conaway, Stowers Institute for Medical Research. Elongin BC-based ubiquitin ligases and transcriptional regulation. (Host: Nouria Hernandez)
Dr. Anirvan Ghosh, Johns Hopkins School of Medicine, Department of Neuroscience. Calcium signaling and the control of cortical connectivity. (Host: Karel Svoboda)

April

Dr. Fred H. Gage, The Salk Institute for Biological Studies, Laboratory of Genetics. Regulation and function of neurogenesis within the adult mammalian brain. (Host: Tony Zador)
Dr. John D. Scott, Howard Hughes Medical Institute/Vollun Institute, Oregon Health & Science University. The molecular architecture of signaling complexes. (Host: Nick Tonks)

October

Dr. Gary Ruvkun, Massachusetts General Hospital, Harvard Medical School. The tiny RNA world. (Host: Winship Herr)
Dr. Morgan Sheng, Massachusetts Institute of Technology. Molecular mechanisms of the dynamic synapse. (Host: Holly Cline)
Dr. Stephen C. Kowalczykowski, Center for Genetics and Development, University of California, Davis. Visualization of recombinational DNA repair at the single molecule level. (Host: Tatsuya Hirano)
Dr. Paul W. Glimcher, Center for Neural Science, New York University. Neural studies of primate decision making. (Host: Zach Mainen)

November

Dr. Ronald DePinho, Dana Farber Cancer Institute, Harvard Medical School. Modeling cancer and cancer genomes. (Host: Terri Grodzicker)
Dr. Ewan Birney, EMBL–EBI, Welcome Trust Genome Campus. Inference of biology from data. (Host: Adrian Krainer)

December

Dr. Mary Ann Osley, Molecular Genetics and Microbiology, University of New Mexico Health Sciences Center. Histone modifications and transcription: The role of histone ubiquitylation. (Host: David Spector)
Dr. Jeffrey Friedman, Howard Hughes Medical Institute, The Rockefeller University. Leptin and the neural circuit regulating body weight and the adaptive response of starvation. (Host: Karel Svoboda)
Dr. Jonathan A. Cooper, Fred Hutchinson Cancer Research Center. Cellular and developmental roles of disabled-related adaptor proteins. (Host: Nick Tonks)

In-House Seminar Program

Cold Spring Harbor In-House Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

January

Yi Zhong: Memory, disease genes, and the fly brain.
Hang Shi (Xu Lab): Structure of the exon-exon junction complex proteins.
Ryohei Yasuda (Svoboda Lab): Imaging the function and plasticity of synaptic channels.
Mingming Zhao (Van Aelst Lab): $p62^{dok}$, a scaffolding protein for negative signaling.

February

Supriya Prasanth (Stillman Lab): ORC, a replication initiator protein required for multiple aspects of cell division cycle.
Kannan Natarajan (Neuwald Lab): Protein kinase structural mechanisms.
Ravi Sachidanandam: Networks: Don't believe the hype?/!

March

Josh Huang: Development of GABAergic circuits in neocortex and cerebellum—In search of a molecular address code for subcellular synapse targeting.
Tony Zador: The cocktail party problem: Computation in the auditory cortex.
Andreas Herbst (Tansey Lab): "Trouble" comes in threes: Conserved elements and their role in *Myc* function.
Eric Julien (Herr Lab): Exploring the chromosome cycle with the herpes simplex virus host-cell factor HCF-1.

April

Ira Hall (Grewal Lab): Role of the RNAi machinery in heterochromatin formation and chromosome dynamics in *S. pombe*.
Susan Janicki (Spector Lab): Silencing to gene expression: Real-time analysis in living cells.

October

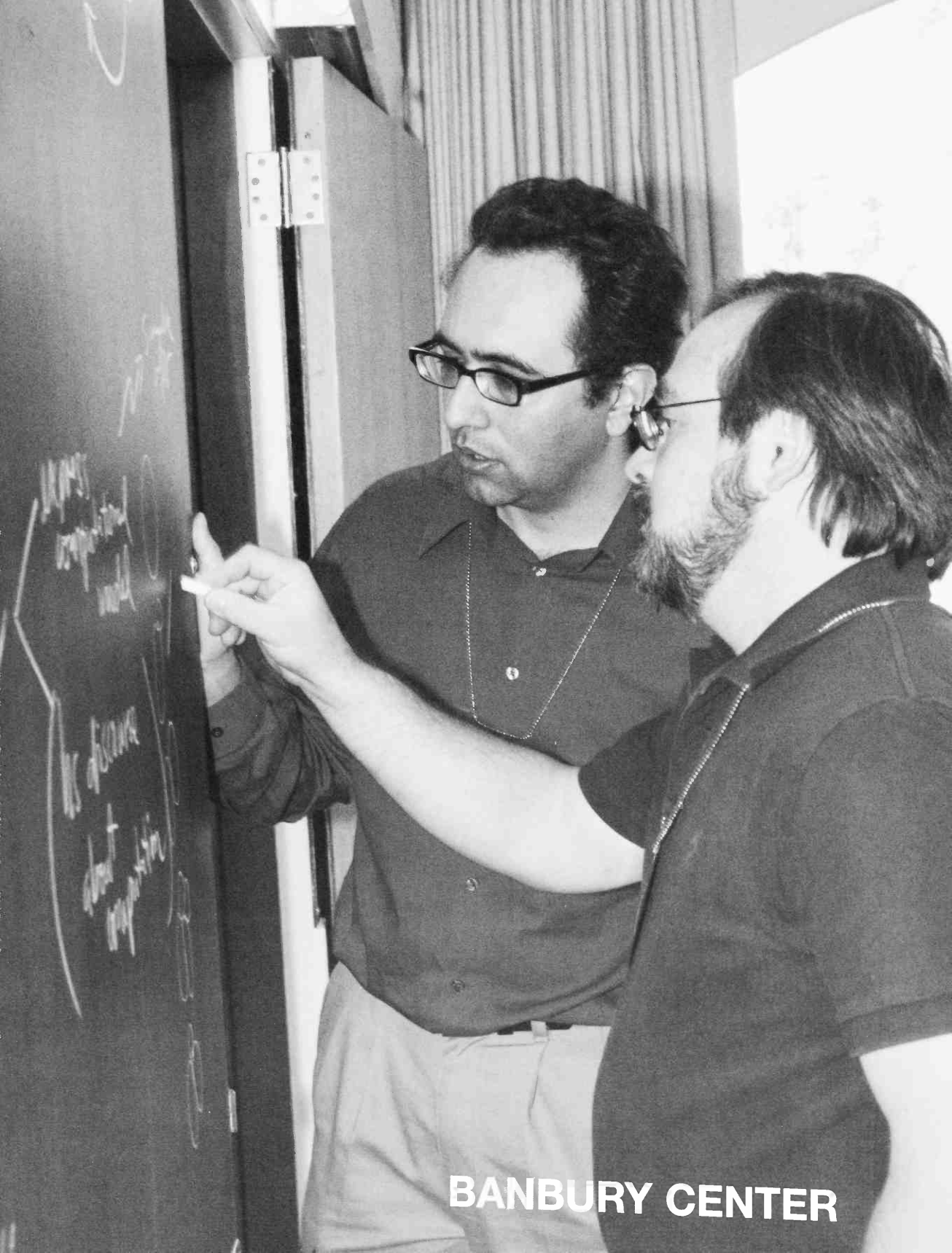
Karel Svoboda: Imaging synaptic plasticity.
Masashi Narita (Lowe Lab): Rb-mediated heterochromatin formation in cellular senescence.
Ajit Janardhan (Skowronski Lab): HIV-1 Nef binds Rac and its upstream activators to alter signaling in T lymphocytes.
Masaaki Hamaguchi: Analysis of *DBC2*, a new tumor suppressor gene.

November

Rob Lucito: ROMA surveying the terrain of cancer genomes.
Fabrice Ango (Huang Lab): Where to put a synapse: Molecular mechanisms of subcellular synapse targeting in cerebellum.
Leemor Joshua-Tor: PAZ pizazz and E1 fun—The single strandedness of it all.

December

Lee Henry: Understanding taste buds at single cell resolution.
Zhenyu Xuan (Zhang Lab): CSHL mammalian promoter database and its applications.
Kurt Haas (Cline Lab): Activity-dependent dendritic arbor growth.


BANBURY CENTER

BANBURY CENTER DIRECTOR'S REPORT

There were 19 meetings at the Banbury Center in 2003, with 654 participants. Of these, 530 (81%) came from the United States, drawn from 33 states. As usual, New York, Maryland, Massachusetts, and California provided most participants (46% of the total), reflecting the many research institutions in those states. Participants from abroad came from 21 countries, once again showing the high esteem in which meetings at Banbury are held throughout the world. The Watson School of Biological Sciences held two week-long "Topics in Biology" courses, and there were five lecture courses in the Laboratory's Meetings and Courses Program. Finally, two local groups made use of the Center. As usual, the program dealt with eclectic, interesting, and often controversial topics. The meetings ranged from discussions of some of the oldest problems in biology, such as *What is a species*?, through theoretical biology, to biomedical topics of the greatest practical importance for many millions of people. There was also one very special event in 2003—Banbury Center's 25th anniversary.

Our 25th Anniversary

That the Banbury Center exists is due to the foresight and wisdom of Charles Robertson, a pivotal figure in the modern development of Cold Spring Harbor Laboratory. He first made a most generous gift of $7.5 million in 1973 to establish the Robertson Fund, the major part of the Laboratory's endowment. Two years later, in 1975, Robertson gifted his Lloyd Harbor estate of some 45 acres and the buildings on it to the Laboratory. These included the family house and a large garage, together with an endowment intended to contribute to the upkeep of the buildings and the estate. It is said that Robertson wanted laboratories built on the site, but this was impractical for financial and esthetic reasons. Instead, Jim Watson suggested that the estate be used for small, workshop-style meetings to complement the large meetings held on the main campus.

Robertson agreed and the garage was converted into a spacious seminar room, with large windows on two sides and a high, vaulted ceiling. These features make our Conference Room so unlike the typical seminar room and its ambience contributes significantly to the success of meetings at Banbury. Another striking feature is the chalkboard across the full width of the room. I can remember Jane Gitschier explaining the polymerase chain reaction to science journalists, beginning at the left side of the board and drawing a diagram that took up all 30 feet of the board. The family house—now named Robertson House—was modified to accommodate meetings participants. (Extra accommodation was provided a few years later when funding from the Max C. Fleischmann and Kresge Foundations was used to build Sammis Hall, and many years later the Meier House was purchased.)

The garage prior to renovations.

The garage during renovations.

The garage today as a conference room.

Cocktails during the 25th Anniversary Party.

D. West, P. Travaglianti, B. Stillman, G. Stillman, K. Friedman

H. Varmus, J. Watson

On June 14, 1977, Francis Crick gave a talk at a small dedication ceremony of what was now called the Banbury Center. Victor McElheny was appointed the first director in early 1978 and Charles Robertson's vision for his family's gift to the Laboratory was properly realized when the first meeting was held in May 1978, on *Assessing Chemical Mutagens: The Risk to Humans*.

We celebrated the Silver Anniversary of that meeting with a small party on September 12. Guests included Bill Robertson and Anne Meier (two of Charles and Marie Robertson's children), former directors Victor McElheny (1978–1982) and Michael Shodell (1982–1986), and friends from Lloyd Harbor. Harold Varmus, Nobel laureate, former director of the National Institutes of Health, and current President of Memorial Sloan-Kettering Cancer Research Center, was the guest of honor. The evening began with cock-

tails in the Conference Room, and was followed by dinner at Robertson House. Dr. Varmus, a frequent participant in Banbury Center meetings, gave a short talk describing the importance of the Banbury Center in promoting biomedical research. It was a delightful evening and made us look forward to what the next 25 years will bring. (In our first 25 years, there were 380 meetings attended by over 11,000 participants.)

Planning and Promoting Biomedical Research

Without exception, all Banbury Center meetings have a significant effect on those participating in them, but there are some meetings that have a demonstrated impact far beyond the confines of the Conference Room. These are fascinating meetings, combining critical reviews of science with discussions of science policy. One example was the 1994 meeting on sequencing the *Arabidopsis* genome that ultimately led to the international effort to complete the sequence. There were similar meetings on three different topics at Banbury in 2003.

L. Joshua-Tor (Cold Spring Harbor Laboratory) and W. Hendrickson (Columbia University) organized the *Scientific Opportunities in Macromolecular Crystallography at NSLS-II* meeting in July. NSLS stands for the National Light Synchrotron Source II that is to be built at Brookhaven National Laboratory. X-ray crystallography is achieving remarkable results in determining the atomic structures of huge molecular complexes. These successes are due to technical advances, not the least of which is the use of very powerful X-rays produced by synchrotrons. This meeting brought together X-ray crystallographers from the relevant institutions to discuss their needs for the NSLS-II. Notable among these was Rod McKinnon, who three months later won the Noble Prize for his work on channel complexes.

We now have the complete sequence of the human genome, but a huge amount of work needs to be done to understand it. A key strategy will be to use other, more tractable experimental animals such as the mouse and the rat. These have different benefits, but the mouse has the edge in terms of its genetics. Mutations are key to doing genetics, and a large number of naturally occurring mouse mutants have been found during the past 100 years. But systematic mutagenesis screens, while expensive and time-consuming to do, have proved invaluable for other organisms, for example, zebrafish. R. Woychik (The Jackson Laboratory) and C. Austin (National Human Genome Research Institute) organized the *Mouse Genome-wide Targeted Mutagenesis* meeting at Banbury to review the scientific and financial issues of carrying out a genome-wide mutagenesis of the mouse. This was an extraordinarily intensive meeting with discussions going on late into the night.

DNA sequence analysis is also being applied to organisms much more esoteric than human and mouse. For many years, taxonomists have used molecular comparisons of proteins and DNA sequences to estimate the relatedness of organisms. Now there is a proposal to use DNA sequences as molecular "bar codes," to use DNA sequences as unique identifiers of species; if a purported new species has the same bar code as one known already, then it is not a new species. The proposal is controversial on scientific grounds—is it right and will it work?—and because it seems to be replacing the skills of the taxonomist with a robotic sequencing machine. The Alfred P. Sloan Foundation funded two meetings to review this proposal. The first meeting, *Taxonomy and DNA*, was organized by R. De Salle (American Museum of Natural History), S. Federhen (National Library of Medicine, National Institutes of Health), and P. Hebert (University of Guelph, Canada). It reviewed some of the fundamental questions: How well do these molecular approaches conform with current methods for defining species? How can molecular data be integrated with other taxonomic data? What are the practical issues involved in carrying this out on a large scale? The second meeting, *Taxonomy, DNA, and the Bar Code of Life*, organized by J. Baker (Academy of Natural Sciences, Philadelphia) and J. Hanken (Harvard University) was primarily a planning meeting, focusing on how to proceed with a museum-based, large-scale DNA bar-coding effort, including strategy, policy, funding, and organization. These remarkable meetings have led to the establishment of a "Bar Code of Life Initiative" Consortium to further these plans.

Plant Science

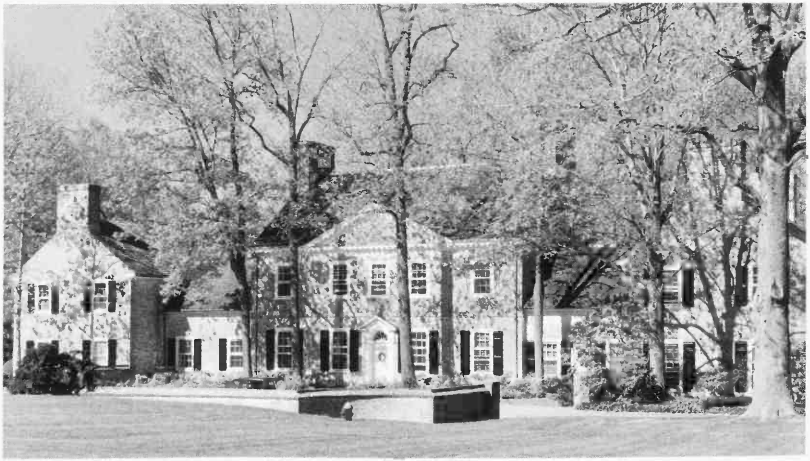
The foundations of developmental biology were laid through observation and experimental manipulation, but now, genomic and comparative genomic approaches are giving us the tools to determine how

limbs or leaves develop. An inflorescence is a cluster of flowers all arising from the same stem, and much work has been done on the genetic basis for inflorescence development in *Arabidopsis*. However, this meeting, *Regulation of Inflorescence Morpholog: Insights from Genetics and Genomics*, organized by E. Kellogg (University of Missouri, St. Louis) and D. Jackson (Cold Spring Harbor Laboratory) and funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program, focused on other plant and nonplant developmental systems. Participants explored how developmental genetics, quantitative trait analysis, and comparative biology can help to discover genes that establish and regulate meristem identity and determine how these gene products interact.

Theoretical Biology

There have been recurring attempts to establish a theoretical biology, in the same way that theoretical physics exists as a well-defined field. These have not been successful—perhaps all kinds of biologists have far too much to discover experimentally to need guidance from theorists. Nevertheless, there are areas where a theoretical approach may well be helpful.

The discussions of species showed how seriously taxonomists take words and descriptions; they aim for unambiguous definitions of a Monarch butterfly or an Indian elephant. Laboratory scientists have been less careful with words and descriptions, using names and concepts that may not have consistent definitions in different research fields. The need for consistency and for new descriptive tools is becoming ever more urgent as the flood of data, and its complexity, continues to increase. The meeting *Formal Languages for Biological Processes*, organized by Y. Lazebnik (Cold Spring Harbor Laboratory), D. Endy (Massachusetts Institute of Technology), and A. Finney (California Institute of Technology) examined some of the approaches being taken to represent biological processes so that they can be more effectively analyzed and understood. Participants were drawn from three areas: experimental biologists who should use these methods, biologists who have successfully used formal approaches, and developers (often mathematicians) of these approaches. The meeting was funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program.



Robertson House provides housing accommodations at Banbury Center.

A second meeting tackling theoretical approaches to biological problems examined some of the simplest gene networks, those of bacteriophage. Organized by S. Adhya (National Cancer Institute), D. Court (National Cancer Institute), and A. Oppenheim (The Hebrew University), *Quantitative Genetic Networks* focused on the quantitative aspects of the genetic and biochemical components underlying the regulatory networks found governing the alternative lifestyles found in bacteriophages. It was remarkable the degree to which even these seemingly simple networks pose problems for formualting theoretical descriptions and devising modern experimental approaches to solve specific complex gene control processes. The meeting was funded by the National Cancer Institute.

Neuroscience

The two Banbury Center meetings on neuroscience also had strong theoretical underpinnings. *Neural Circuits: Principles of Design and Operation* was organized by K.D. Miller (University of California, San Francisco) and H.S. Seung (Massachusetts Institute of Technology). Funded by the Swartz Foundation, the meeting brought together experimentalists and theoreticians working on operation of neuronal circuits. The participants considered results on cortical circuitry, compared and contrasted results from visual, somatosensory, and auditory cortex and the bird song system, and considered theoretical and engineering approaches to the construction of neural circuits.

The second meeting, *Neural Representation and Processing of Temporal Patterns*, was organized by C. Brody (Cold Spring Harbor Laboratory), D. Buonomano (University of California, Los Angeles), and J. Hawkins (Redwood Neuroscience Institute). Processing of temporal patterns is a fundamental component of sensory and motor function. Given the inherent temporal nature of our interaction with the environment, understanding how the brain processes time is a necessary step toward understanding the brain. Participants considered the following fundamental problems: How is time represented in the brain? How are complex temporal patterns perceived and produced? Participants included psychologists, neuroscientists, and theorists working on these problems, and the meeting was funded by the Redwood Neuroscience Institute.

Human Neurological and Neurodegenerative Disorders

Three meetings dealt with neurological and neurodegenerative disorders. A fourth meeting dealt with stem cells and is included here, given that stem cells may prove to be effective in neurodegenerative diseases.

Banbury has regularly held meetings on Fragile-X syndrome, most recently funded by the FRAXA Research Foundation. Research on Fragile-X is at a most interesting stage, with evidence that abnormalities may exist in the formation of contacts between nerve cells. This is generating a large amount of intense research which was reviewed in the meeting *Synaptic Function in Fragile-X*, organized by M. Bear (Brown University) and M. R. Tranfaglia (FRAXA Research Foundation). It focused on the biological activity of the FMRP protein and on its role in dendrite formation. Most importantly, understanding the mechanisms involved will lead to identifying other components which may be therapeutic targets.

Research on amyotrophic lateral sclerosis (ALS)—the most common form of motor neuron diseases—is also at a most interesting stage. From 5% to 10% of ALS is inherited, and in 20% of these families, there are mutations in the enzyme Cu/Zn Superoxide dismutase 1. This discovery made a major impact on the field, but the mechanism of SOD1 toxicity remains unclear and the cause(s) of the remaining ALS cases is unknown. These latter cases were the subject of *Finding New Genes Linked to Amyotrophic Lateral Sclerosis: A Focus on Current Technologies and Their Potential Application*, organized by R. Brown (Massachusetts General Hospital) and L. Bruijn (The ALS Association) and funded by the ALS Association. It examined how the complete human genome sequence and new technology advances can be used to look for the genetic components of these unknown cases. The discovery of new genes, either linked to familial disease or susceptibility genes in the sporadic cases, is critical for a better understanding of the disease and the potential for new therapeutic targets.

Banbury Center held a meeting on schizophrenia for the first time since the early 1990s. Since then, several areas of the genome have been suspected of harboring genes contributing to schizophrenia, but no gene has yet been pinned down. On the other hand, there have been advances in the neuropharmacology of schizophrenia, particularly in the role of a molecule called NMDA. Participants in this meeting, *Integrating Progress in the Genetics and Neuropharmacology of Schizophrenia*, organized by R. Cloninger (Washington University) and J. Coyle (Harvard University), attempted to bring together both areas of research. The goals of the meeting were to identify new technical and experimental advances in the fields of genetics, cognitive development, brain imaging, and neuropharmacology that might be applied to understanding schizophrenia. The meeting was funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program.

No area of contemporary biomedical research has been more controversial than studies of human stem cells. The promise of using stem cells for therapy is great, but much still must be learned about the biology of these remarkable cells. R. McKay (National Institute of Neurological Disorders and Stroke) organized a meeting, *Controlling the Differentiation of Pluripotent Cells*, that examined how these cells differentiate into many cell types, and the ways in which this differentiation might be directed. A key question is the extent to which work on other organisms, in particular the mouse, can be extrapolated to human cells. The ethical implications of this work were not ignored; there was an energetic discussion of some of the philosophical underpinnings of people's reactions to human stem cell research. The meeting was funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program.

Cancer

The meetings on cancer in 2003 all concentrated on clinical issues, rather than on research on the fundamental causes of cancer.

Two meetings dealt with very interesting forms of cancer. The first was on *The Biology of Neuroendocrine Tumors*, organized by A. Levine (Institute for Advanced Studies), and E. Vosburgh (Verto Institute), and funded by the Verto Institute. These carcinoid and other neuroendocrine tumors are derived from cells that share neural and endocrine features. They have a unique biology that determines the clinical features of the tumors and that might be exploited in developing treatments. These treatments might be very different from those currently available for other cancers. This meeting provided an opportunity to bring together current and future Verto investigators, and the Scientific Advisors of the Verto Institute.

The second meeting dealt with a related group of unusual cancers, pheochromocytomas, which secrete catecholamines and cause hypertension. *Molecular Differentiation of Benign and Malignant Pheochromocytomas and Neuroblastomas*, organized by G. Eisenhofer (National Institutes of Health), W.M. Manger (National Hypertension Association, Inc.), and R.M. Weinshilboum (Mayo Foundation), was funded by the National Hypertension Association, Inc. There are no reliable prognostic markers to assess whether a pheochromocytoma will metastasize—presumably these differences in behavior reflect the underlying mutations and differences in expression of genes regulating cellular growth and survival. An understanding of these pathways of gene expression should therefore allow identification of molecular markers of malignancy and possible new targets for therapeutic intervention. Participants reviewed current methods for distinguishing benign from malignant forms of pheochromocytoma and went on to survey the use of microarrays and other genomics-based tools for detecting malignancy in these types of tumor.

Finally, T. Kreiner (Affymetrix, Inc.), T. Golub (Whitehead Institute for Biomedical Research), and D. Dalma-Weiszhausz (Affymetrix, Inc.) organized a meeting, *Taking Cancer Genomics to the Clinic*, funded by Affymetrix, Inc. This was a high-level discussion workshop that examined how genomic approaches to cancer will promote the implementation of new techniques and strategies. In particular, participants reviewed the current diagnostic and prognostic clinical practices in the treatment of cancer and how techniques such as microarrays might change them. Participants included cancer researchers, clinicians, policy-makers, and members of advocacy groups, and an important goal of the meeting was to strengthen the relationships in the cancer world among advocacy groups, researchers, policy-makers, the FDA, and technology providers.

Infectious Diseases and Chronic Disorders

Toward a More Unified Understanding of Infectious Disease, organized by V. McGovern (Burroughs Wellcome Fund) and S. James (The Ellison Medical Foundation), and funded by the Burroughs Wellcome Fund, reviewed the current state of basic research in the human pathogens. After surveying the impact and potential of genomic and postgenomic approaches, participants went on to consider where infectious diseases research is going, and how a more comprehensive understanding of the interface between pathogens and host can be gained. One goal of the meeting was to explore the desirability of integrating research across the pathogens, and the difficulties involved in taking this broader approach. It is a sign of the times that veterinary pathogens were included in the discussions.

Infectious pathogens were also part of the meeting *Toward Understanding the Cellular and Molecular Mechanisms of Medically Unexplained Fatigue*. This topic is highly controversial precisely because it is medically unexplained and so, in the view of many, such fatigue does not exist except in the mind of the individual. For this reason, the organizers, W.C. Reeves and S.D. Vernon (Centers for Disease Control & Prevention) and C. Heim (Emory University), covered a remarkably wide range of topics, from infections and toxins, to how the mind influences onset and recovery. Funded by the Centers for Disease Control & Prevention, it was a thought-provoking meeting that included discussions of mind/body relationships.

Vaccines

Banbury Center has hosted a series of colloquia sponsored by the Albert B. Sabin Vaccine Institute on key issues limiting the production and distribution of vaccines, particularly to the most disadvantaged populations. The 2002 colloquium, *Global Vaccine Shortage: The Threat to Children and What to Do About It*, identified the four priority issues for which feasible solutions could be found: Stockpiling,

Meier House Provides housing accommodations for meeting participants at Banbury Center.

International Commission on Harmonization (ICH) on Vaccines, Financing, and Public Advocacy. L. Miller (Intermedica, Inc.) and N. Tomich (U.S. Medicine Institute) convened the 2003 colloquium, *Feasible Solutions to Global Vaccine Shortages*, to propose ways to tackle these issues. The meeting was funded by the Albert B. Sabin Vaccine Institute through a grant from the Bill and Melinda Gates Foundation.

Eugenics

The Ethical, Legal, and Social Issues (ELSI) division of the National Human Genome Research Institute provided funds for *An Image Archive of the American Eugenics Movement* (Dolan DNA Learning Center). Part of the grant was to be used for meetings introducing opinion leaders and policy-makers from government, science, healthcare, education, and the mass media to this episode in American social history. The meeting in 2003 was the last in this series. Organized by D. Micklos (Dolan DNA Learning Center) and myself, the emphasis of the meeting changed a little so as to include contemporary issues. *Eugenics, Genes, and Human Behavior* ranged from a presentation on Sir Francis Galton, through the eugenics movement of 1910–1940, to present-day efforts to find genes affecting human behavior.

Watson School of Biological Sciences

Of the two Topics in Biology Courses for students in the Watson School, the first was a repeat of the course on *Evolution* taught by N. Patel (University of Chicago). The second course was a new one on *Animal Behavior*, taught by H.K. Reeve from Cornell. This, like the others in the program, was tremendously successful, with students observing the behavior of solitary wasps on the Banbury estate, as well as the behavior of larger animals at the Bronx Zoo.

Acknowledgments

The continuing success of the Banbury Center program is due to the efforts of many people: Bea Toliver and Ellie Sidorenko in the Banbury office, Katya Davey at Robertson House, and Chris McEvoy, Joe Ellis, and Danny Maxfield looking after the grounds. All worked very hard to keep the Center running. Food and Beverage Services, Audiovisual, Housekeeping Services, and the Meetings Office had key roles in the smooth operation of the Center. The meetings could not take place without the hard work of the organizers, the generosity of the Laboratory's Corporate Sponsors, and the other donors who funded our meetings, and the Laboratory's scientists who continue to support the Center.

Jan Witkowski

MEETINGS

Toward Understanding the Cellular and Molecular Mechanisms of Medically Unexplained Fatigue

February 23–26

FUNDED BY **Centers for Disease Control & Prevention and The CFIDS Association of America**

ARRANGED BY **W.C. Reeves,** Centers for Disease Control & Prevention, Atlanta, Georgia
S.D. Vernon, Centers for Disease Control & Prevention, Atlanta, Georgia
C. Heim, Emory University School of Medicine, Atlanta, Georgia

Introduction: S.D. Vernon, Centers for Disease Control & Prevention, Atlanta, Georgia

SESSION 1: Setting the Stage: Chronic Fatigue and the State-of-the-Science
Chairpersons: B. Evengard, Karolinska Institutet, Stockholm, Sweden; **U. Vollmer-Conna,** University of New South Wales, Sydney, Australia

S. Wessely, Institute of Psychiatry, London, United Kingdom: The spectrum of ailments in medically unexplained fatiguing illnesses and why a multidisciplinary, integrated approach is necessary to further our understanding.

W.C. Reeves, Centers for Disease Control & Prevention, Atlanta, Georgia: Conservative estimates of the magnitude of medical unexplained fatigue.

P.D. White, St. Bartholomew's Hospital, London, United Kingdom: Cognitive, behavioral, and emotional factors in chronic fatigue.

D. Papanicolaou, Emory University School of Medicine, Atlanta, Georgia: Neuroendocrine perturbations in chronic fatigue.

A. Lloyd, University of New South Wales, Sydney, Australia: Acute infection, immunologic perturbations, and chronic fatigue.

SESSION 2: Influences on the Structure and Function of the Brain
Chairpersons: E.R. Unger, Centers for Disease Control & Prevention, Atlanta, Georgia;
A.H. Miller, Emory University School of Medicine, Atlanta, Georgia

J.C. de la Torre, University of California, San Diego, Escondido: Cerebral perfusion and neurometabolic-synaptic activity in normal and abnormal states: Relevance to CFS?

C.D. Sladek, University of Colorado Health Sciences Center, Denver: Regulation of the neurohypophyseal system: Neurotransmitter, neuropeptide, and steroid hormone interactions.

S. Vernon

SESSION 3: Infection, Immunity, Sex, and the Brain
Chairperson: M.A. Fletcher, University of Miami School of Medicine, Florida

E. M. Sternberg, National Institute of Mental Health, Bethesda, Maryland: Neuroendocrine regulation of immunity.
P.H. Patterson, California Institute of Technology, Pasadena: Maternal infection, fetal brain development, and health.
I. Hickie, St. George Hospital, Sydney, Australia: Persistent infection and immunity and chronic consequences.

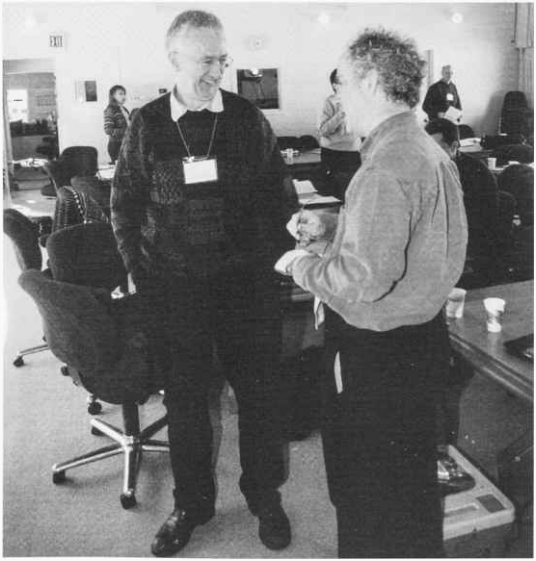
SESSION 4: In Search of a Marker: Analytical Approaches Applicable to Medically Unexplained Fatigue
Chairpersons: K. Kenney, The CFIDS Association of America, Charlotte, North Carolina;
E. Hanna, National Institutes of Health, Bethesda, Maryland

A.J. Cleare, Guy's, King's and St. Thomas' School of Medicine, London, United Kingdom: What pyschopharmacology tells us about the pathophysiology of medically unexplained fatigue.
S. Vernon, Centers for Disease Control & Prevention, Atlanta, Georgia: Biomarker discovery in illness with no lesion.
S. Shriver, Penn State University, University Park: X versus Y: Sex-based disease differences.
C. Artlett, Thomas Jefferson University, Philadelphia, Pennsylvania: Microchimerism: Incidental by-product of pregnancy or active participant in human health?

SESSION 5: Can We Explain What Might be Happening in the Brain?
Chairperson: J.F. Jones, National Jewish Medical & Research Center, Denver, Colorado

Workshop Committee Chairperson: E.R. Unger, Centers for Disease Control & Prevention, Atlanta, Georgia

Summary Report: Future Research Directions



P. White, S. Wessely

Taxonomy and DNA

March 9–12

FUNDED BY **Alfred P. Sloan Foundation**

ARRANGED BY **R. DeSalle,** American Museum of Natural History, New York
S. Federhen, National Library of Medicine, NIH, Bethesda, Maryland
P. Hebert, University of Guelph, Ontario, Canada

SESSION 1: Species: Biology's Fundamental Particles

Key Questions for Session

N. Knowlton, University of California, San Diego: Marine biodiversity: Marrying DNA and natural history.
B. Golding, McMaster University, Hamilton, Canada: The nature of substitutions that distinguish species.
K.L. Shaw, University of Maryland, College Park: DNA variation in newly evolved species.
J. Hanken, Harvard University, Cambridge, Massachusetts: Cryptic biodiversity: Molecular and morphological approaches.

Discussion of Session

Comments by Discussion Leaders: G. Caccone, Yale University, New Haven, Connecticut; **G. Amato,** Wildlife Conservation Society, Bronx, New York

SESSION 2: Probing Life's Diversity with DNA

Key Questions for Session

N. Pace, University of Colorado, Boulder: The large-scale structure of the Tree of Life.
M. Sogin, The Marine Biological Society, Woods Hole, Massachusetts: Molecular evolution of eukaryotes: A protist perspective.
P. Hebert, University of Guelph, Ontario, Canada: Microgenomics and animal diversity.

Discussion of Session

Comments by Discussion Leaders: K. de Queiroz, Smithsonian Institution, Washington, D.C.; **A. Vogler,** The Natural History Museum, London, United Kingdom

SESSION 3: Large-scale Biology I
R. McCombie, Cold Spring Harbor Laboratory: Genome projects: A PI's perspective.

SESSION 4: The Analytical Engines

Key Questions for Session

D. Hillis, University of Texas, Austin: The Tree of Life and its role in automated species identification.
K. Crandall, Brigham Young University, Provo, Utah: Bioinformatics methods for large-scale phylogenetics.
R. DeSalle, American Museum of Natural History, New York: Phylogenetic systematics and diagnosis.

Discussion of Session

Comments by Discussion Leaders: A. Bucklin, University of New Hampshire, Durham; **B. Schierwater,** ITZ, Ecology of Evolution, Hanover, Germany

G. Caccone, K. Shaw, K. Crandall

SESSION 5: Collections of Life

Key Questions for Session

B. Thiers, New York Botanical Garden, Bronx, New York: Herbaria and systematics: Past, present, and future.
S. Federhen, National Library of Medicine, NIH, Bethesda, Maryland: Linking sequences to specimens.
M. O'Leary, Stony Brook University, New York: MorphoBank.
D. Janzen, University of Pennsylvania, Philadelphia: Reflections on large-scale biodiversity inventories.

Discussion of Session

Comments by Discussion Leaders: W. Hallwachs, University of Pennsylvania, Philadelphia; **M. Stoeckle,** Cornell University Medical College, Ithaca, New York

SESSION 6: Large-scale Biology II

N. Zinder, The Rockefeller University, New York: Genome projects: Money and politics.

SESSION 7: Group Discussions

Introduction to Purpose

Group 1: Acquiring Specimens for DNA Analysis and Acquiring DNA Sequences
Leaders: F. Grassle, Rutgers University, New Brunswick, New Jersey; **K.L. Shaw,** University of Maryland, College Park

Group 2: Analyzing the DNA Sequences and Project Delineation
Leaders: D. Hickey, University of Ottawa, Ontario, Canada; **R. DeSalle,** American Museum of Natural History, New York

Reports and Discussion
Chairperson: P. Hebert, University of Guelph, Ontario, Canada

M. Sogin, J. Ausubel

Quantitative Genetic Networks

March 16–19

FUNDED BY **Center for Cancer Research, National Cancer Institute**

ARRANGED BY **S. Adhya,** National Cancer Institute, NIH, Bethesda, Maryland
D.L. Court, National Cancer Institute, Frederick, Maryland
A. Oppenheim, Hebrew University, Hadassah Medical School, Jerusalem, Israel

SESSION 1: λ: CII and CIII
Chairperson: A.M. Campbell, Stanford University, California

A.M. Campbell, Stanford University, California: What do we know—not know about CII?
A. Oppenheim, Hebrew University, Hadassah Medical School, Jerusalem, Israel: CII and CIII in the genetic networks.
P. Parrack, Bose Institute, Kolkata, India: CII stability and transcriptional control: Structural insights and functional implications.
R. Weiss, Princeton University, New Jersey: Rational design and directed evolution strategies for constructing synthetic gene networks.
S. Adhya, National Cancer Institute, NIH, Bethesda, Maryland: λ's lifestyle decision: Stochastic or deterministic?

SESSION 2: λ: CI and Cro
Chairperson: S. Adhya, National Cancer Institute, NIH, Bethesda, Maryland

G. Gussin, University of Iowa, Iowa City: Quantitative aspects of regulation of the PRM promoter.
J.W. Little, University of Arizona, Tucson: Genetic circuitry: Are its features essential or refinements to a basic ground plan?
I. Dodd, University of Adelaide, Australia: Role of Cro in the bistable switch.
S.L. Svenningsen, University of Copenhagen, Denmark: Insights into the role of Cro and OL in regulation of PR.
K. Shearwin, University of Adelaide, Australia: The long-range interaction between OL and OR.

SESSION 3: λ: Genetics Circuitry and Modeling
Chairperson: M. Ptashne, Memorial Sloan-Kettering Cancer Center, New York

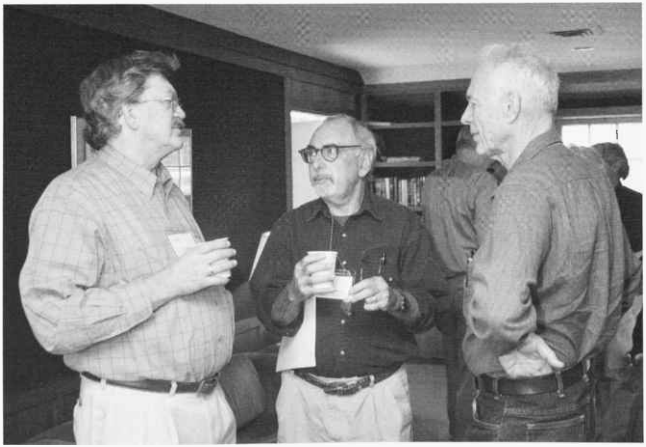
S. Brown, University of Copenhagen, Denmark: Single cell analysis of phophage stability. S. Roy, Bose Institute, Calcutta, India: CI property: A new look at the genetic switch.
K. Sneppen, NORDITA, Copenhagen, Denmark: Modeling OL-OR derepression and stability.
M.B. Elowitz, The Rockefeller University, New York: Synthetic gene networks and genetic noise in cells.
D.L. Court, National Cancer Institute, Frederick, Maryland: Effect of genes from PL operon on everything else.

SESSION 4: Other Aspects of λ
Chairperson: H. Eisen, Seattle, Washington

M.E. Gottesman, Columbia University, New York: Exclusion of λ by phage HK022.
L. Thomason, National Cancer Institute, Frederick, Maryland: Role of *rex* and *ren* genes.
R. Young, Texas A&M University, College Station: Lysis timing: Diverse solutions to the paramount regulatory problem.
J.R. Roth, University of California, Davis: Effect of prophage on induction of the SOS system and the activity of RecA co-protease.
J. Stavans, Weizmann Institute of Science, Rehovot, Israel: The SOS response of single cells.

SESSION 5: Other Phages
Chairperson: A. Das, University of Connecticut, Farmington

L. Orosz, Eotvos Lorand University, Budapest, Hungary: Rhizobium phage 16-3: DNA looping; two immunity regions.
E. Haggard, Stockholm University, Sweden: Comparison of the genetic switches in P2-related phages.
K. Hammer, BioCentrum–DTU, Lyngby, Denmark: The genetic switch in lactococcal phage TP901-1.
R. d'Ari, Institut Jacques Monod, Paris, France: Does phage Mu *gemA* gene affect cell cycle?
D. Endy, Massachusetts Institute of Technology, Cambridge: Phage T7.



J. Roth, M. Gottesman, A. Campbell

Toward a More Unified Understanding of Infectious Disease

March 23–26

FUNDED BY **Burroughs Wellcome Fund**

ARRANGED BY **V. McGovern,** Burroughs Wellcome Fund, Research Triangle Park, North Carolina
S.L. James, The Ellison Medical Foundation, Bethesda, Maryland

Opening Remarks: **V. McGovern,** Burroughs Wellcome Fund, Research Triangle Park, North Carolina

SESSION 1: Overview

E.C. Bond, Burroughs Wellcome Fund, Research Triangle Park, North Carolina

SESSION 2: Current Needs and Expectations: Pathogenesis Research and the Development of Vaccines/Drugs/Diagnostics

A. Mahmoud, Merck & Co., Inc., Whitehouse Station, New Jersey

SESSION 3: Current Research Perspectives: "Classical" Infectious Diseases

G.I. Miller, Jr., Yale University School of Medicine, New Haven, Connecticut
K. Haldar, Northwestern University Feinberg, Chicago, Illinois
A. Casadevall, Albert Einstein College of Medicine, Bronx, New York
P. Small, University of Tennessee, Knoxville

SESSION 4: Current Research Perspectives: Infectious Roots of Chronic Diseases

M.J. Blaser, New York University School of Medicine, New York
R.H. Yolken, John Hopkins University, Baltimore, Maryland
M.V. Pletnikov, Johns Hopkins University School of Medicine, Baltimore, Maryland

SESSION 5: Group Discussion: What Are The Current Expectations for Infectious Disease Research?

Discussion Leader: G.I. Miller, Jr., Yale University School of Medicine, New Haven, Connecticut

SESSION 6: What We Heard—Summary of First Day

Discussion Leader: G.I. Miller, Jr., Yale University School of Medicine, New Haven, Connecticut

SESSION 7: What Could We Gain from a Broader Perspective: Successes and Barriers

N.M. Agabian, University of California, San Francisco
J. Heitman, Duke University, Durham, North Carolina
F. Sher, NIAID, National Institutes of Health, Bethesda, Maryland
M.K. Hostetter, Yale University School of Medicine, New Haven, Connecticut
T.L. Doering, Washington University School of Medicine, St. Louis, Missouri
J.H. McKerrow, University of California, San Francisco

SESSION 8: Group Discussion

J. Heitman, Duke University Medical Center, Durham, North Carolina
F. Sher, NIAID, National Institutes of Health, Bethesda, Maryland

SESSION 9: What's in the Toolbox?

G.M. Weinstock, Baylor College of Medicine, Houston, Texas
T.B. Kepler, Duke University, Durham, North Carolina
P. Kelling, University of British Columbia, Vancouver, B.C., Canada
M.-W. Tan, Stanford University, California

SESSION 10: Group Discussion

Discussion Leader: K. Haldar, Northwestern University Feinberg School of Medicine, Chicago, Illinois

R. Rabinovich, Bill & Melinda Gates Foundation, Seattle, Washington: Hopes and aspirations of the Bill & Melinda Gates Foundation.

SESSION 11: What We Heard

Discussion Leaders: F. Sher, NIAID, National Institutes of Health, Bethesda, Maryland and **K. Haldar,** Northwestern University Feinberg School of Medicine, Chicago, Illinois

SESSION 12: Putting the Ideas into Action—What Can Funders Do?

P. Sager, National Institute of Allergy & Infectious Diseases, Bethesda, Maryland
W.R. Galey, Howard Hughes Medical Institute, Chevy Chase, Maryland
V. McGovern, Burroughs Wellcome Fund, Research Triangle Park, North Carolina
S.L. James, The Ellison Medical Foundation, Bethesda, Maryland

SESSION 13: Group Discussion

A. Mahmoud, Merck & Co., Inc., Whitehouse Station, New Jersey
S.L. James, The Ellison Medical Foundation, Bethesda, Maryland

F. Sher, E. Bond, A. Mahmond

Synaptic Function in Fragile-X

March 30–April 2

FUNDED BY **National Institute of Mental Health, NIH, through a grant to FRAXA Research Foundation**

ARRANGED BY **M.F. Bear,** Howard Hughes Medical Institute, Brown University, Providence, Rhode Island
M.R. Tranfaglia, FRAXA Research Foundation, Newburyport, Massachusetts

Opening Remarks: **M.R. Tranfaglia,** FRAXA Research Foundation, Newburyport, Massachusetts and **K. Clapp,** FRAXA Research Foundation Newburyport, Massachusetts: Patient/clinical perspective
M.F. Bear, Howard Hughes Medical Institute, Brown University, Providence, Rhode Island: An mGluR hypothesis of mental retardation

SESSION 1: Dendritic RNA Protein Synthesis and Structural Plasticity
Chairperson: W.T. Greenough, University of Illinois, Urbana

J. Yin, Cold Spring Harbor Laboratory: Synaptic tagging hypothesis applied to Fragile-X.
G.J. Bassell, Albert Einstein College, Bronx, New York: Regulation and mechanism of FMRP trafficking in dendrites.
O. Steward, University of California, Irvine: Targeting mRNA to synaptic sites on dendrites.
J.R. Fallon, Brown University, Providence, Rhode Island: Translational regulation of the Fragile-X message.

SESSION 2: Dendritic RNA Protein Synthesis and Structural Plasticity (Continued)
Chairperson: M.F. Bear, Howard Hughes Medical Institute, Brown University, Providence, Rhode Island

I.J. Weiler, University of Illinois, Urbana-Champaign: Regulation of protein synthesis at the synapse.
K.M. Huber, University of Texas Southwestern Medical Center, Dallas: Effects of mGlR1 and MGluR5 antagonists on long-term depression in hippocampal area CA1.
P.W. Vanderklish, Scripps Research Institute, La Jolla, California: Relationships between synaptic structure and dendritic translation.
W.T. Greenough, University of Illinois, Urbana: Structural consequences of the absence of FMRP.
B. Oostra, Erasmus Universiteit Rotterdam, The Netherlands: Role of FMRP in the dendrite.
E. Khandjian, Hospital St. Francois dAssise, Universite Lavel, Quebec, Canada: Models of Fragile-X syndrome.

D. Stevenson, A. Caudy, M.A. Busby

SESSION 3: mGluRs
Chairperson: K.M. Huber, University of Texas Southwestern Medical Center, Dallas

R.K.S. Wong, Stony Brook University, New York, Health Science Center, Brooklyn: Group I metabotropic glutamate-receptor-mediated epileptogenesis in the cortex.

R.P. Bauchwitz, St. Luke's–Roosevelt Institute of Health Sciences, Columbia University, New York: Effects of the mGluR antagonist MPEP in the fmr1-tm1Cgr Fragile-X mouse.

W. Spooren, Hoffman-La Roche, Basel, Switzerland: Behavioral pharmacology of mGlu5 receptor antagonists: Implications for the treatment of anxiety disorders.

F. Gasparini, Novartis Pharma AG, Basel, Switzerland: Discovery and characterization of ligands for the group I mGluRs: Agonists, antagonists, allosteric positive modulators, allosteric negative modulators, neutral ligands.

SESSION 4: Ampa Receptor Regulation
Chairperson: O. Steward, University of California, Irvine

R. Malinow, Cold Spring Harbor Laboratory: AMPA receptor trafficking and synaptic plasticity.

M.F. Bear, Howard Hughes Medical Institute, Brown University, Providence, Rhode Island: AMPA receptor regulation during hippocampal LTD.

D.J. Linden, Johns Hopkins University School of Medicine, Baltimore, Maryland: Molecular basis of cerebellar long-term synaptic depression.

P.L. Carlen, Toronto Western Hospital, Canada: Reduced cortical, but no hippocampal LTP, in the FMR1 gene knockout mouse is associated with decreased expression of the cortical GluR1.

J.R. Larson, University of Illinois, Chicago: Olfactory learning and memory in a mouse model for Fragile-X.

E. Berry-Kravis, Rush Children's Hospital, Toronto, Canada: AMPA receptor activator (Ampakine) CX516 and Fragile-X syndrome.

SESSION 5: Biology of FMRP
Chairperson: I.J. Weiler, University of Illinois, Urbana

S.T. Warren, Howard Hughes Medical Institute, Emory University School of Medicine, Atlanta, Georgia: Introduction to the biology of FMRP.

J. Darnell, The Rockefeller University, New York: RNA targets of the FMRP protein.

A. Beckel-Mitchener, Beckman Institute, Urbana, Illinois: FMRP target mRNAs and their potential functions.

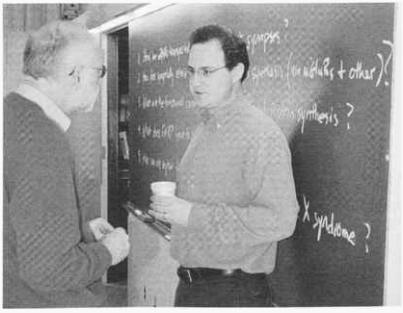
D. Zarnescu, Emory University, Atlanta, Georgia: Penelope is the major autosomal modifier of *Drosophila* Fmr1 overexpression in the eye.

A. Caudy, Cold Spring Harbor Laboratory: *Drosophila* Fragile-X-related protein associates with the RNA interference machinery.

D.L. Nelson, Baylor College of Medicine, Houston, Texas: Developing phenotypic assays in mouse and fly models of deficiencies in FMR1 and related genes.

SESSION 6: Conclusion

M.F. Bear, Howard Hughes Medical Institute, Brown University, Providence, Rhode Island and W.T. Greenough, University of Illinois, Urbana: Wrap-up and discussion



B. Oostra, G. Bassell

D. Nelson, S. Warren

Taking Cancer Genomics to the Clinic

April 13–15

FUNDED BY **Affymetrix, Inc.**

ARRANGED BY **T. Kreiner,** Affymetrix, Inc., Santa Clara, California
T. Golub, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
D. Dalma-Weiszhausz, Affymetrix, Inc., Santa Clara, California

SESSION 1: State of Cancer Genomics

T. Kreiner, Affymetrix, Inc., Santa Clara, California: Background of meeting.
P. Meltzer, National Human Genome Research Institute, NIH, Bethesda, Maryland: Translational implications of expression profiling in sarcomas and breast cancer.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introductory remarks.
T. Golub, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Overview of the meeting.

SESSION 2: Current State of Cancer Genomics
Chairperson: T. Golub, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

P. Meltzer, National Human Genome Research Institute, NIH, Bethesda, Maryland: Introduction.
L.M. Staudt, National Cancer Institute, Bethesda, Maryland: Molecular diagnosis and outcome prediction in lymphoid malignancies by gene expression profiling.
J.R. Downing, St. Jude Children's Research Hospital, Memphis, Tennessee: Gene expression profiling in acute leukemias: Clinical applications.
S. Ramaswamy, Dana-Farber/Whitehead, Cambridge, Massachusetts: Multiclass molecular diagnosis and staging of cancer patients.

SESSION 3: Technology Platform
Chairperson: W.H. Koch, Roche Molecular Systems, Alameda, California

W.H. Koch, Roche Molecular Systems, Alameda, California: Introduction.
J. Baker, Genomic Health, Inc., Redwood City, California: Determination of tumor gene expression profiles using fixed paraffin-embedded specimens.
R. Carlson, Vysis Inc., Downers Grove, Illinois: Application of genomic microarrays and FISH to disease management.
T. Orntoft, Aarthus University Hospital, Denmark: Classification of bladder cancer using gene expression.
M. Rubin, Brigham and Women's Hospital, Boston, Massachusetts: Development of a prostate cancer biomarkers to monitor disease progression.
J. Warrington, Affymetrix, Inc., Santa Clara, California: Array-based treatment management assays.
E.F. Petricoin, Food and Drug Administration, Bethesda, Maryland: Serum proteomic pattern diagnostics: Moving to clinical applications.

SESSION 4: FDA Perspectives

E. Mansfield, Food and Drug Administration, Rockville, Maryland: FDA/CDRH Office of in vitro devices: New draft guidance for microarrays and multiplex tests.

SESSION 5: Clinical Trials and Validation Challenges
Chairperson: T.J. Triche, Children's Hospital, Los Angeles, California

T.J. Triche, Children's Hospital, Los Angeles, California: Introduction.
A.J. Buckler, Ardais Corporation, Lexington, Massachusetts: Setting standards for clinical genomics in ostic and therapeutic discovery and development.
J.M. Olson, Fred Hutchinson Cancer Research Center, Seattle, Washington: New therapies based on genomic studies.
M. van de Vijver, The Netherlands Cancer Institute, Amsterdam: Gene expression profiling to predict outcome in breast cancer.
E. Feigal, National Cancer Institute, Bethesda, Maryland: NCI initiatives to accelerate movement of genomics to the clinic.

SESSION 6: Review and Summary

Discussion Leaders: T. Kreiner, Affymetrix, Inc., Santa Clara, California; **T. Golub,** Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

Formal Languages for Biological Processes

April 20–23

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **D. Endy,** Massachusetts Institute of Technology, Cambridge
A. Finney, University of Hertfordshire, Herts, United Kingdom
Y. Lazebnik, Cold Spring Harbor Laboratory

SESSION 1: Do We Need Formal Representations of Biological Systems?
Chairperson: D. Eddy, Massachusetts Institute of Technology, Cambridge

R. Brent, The Molecular Sciences Institute, Berkeley, California: Natural language representations of intracellular biological processes.
M. Wigler, Cold Spring Harbor Laboratory: Say What?
F. Cross, The Rockefeller University, New York: Cell cycle control: Complex, or just complicated?
P.A. Silver, Dana Farber Cancer Institute, Boston, Massachusetts: Connecting the genome to the cytoplasm.

General Discussion: Challenges Facing Useful Representations

SESSION 2: Biological Systems and Representations Thereof I
Chairperson: Y. Lazebnik, Cold Spring Harbor Laboratory

R. Iyengar, Mount Sinai Medical Center, New York: The activity of biochemical signaling networks and the origins of spatial domains.
D. Chklovskii, Cold Spring Harbor Laboratory: Search for multineuron modules in *C. elegans* brain.
S.J. Wodak, Universite Libre de Bruxelles, Brussels, Belgium: Representing and analyzing molecular interactions and cellular processes.
K.W. Kohn, National Cancer Institute, Bethesda, Maryland: Representation of bioregulatory networks and the origins of spatial domains.
R. Maimon, Gene Network Sciences, Ithaca, New York: Diagrammatic notation and computation grammar for gene networks.

General Discussion: Limits of Current Representations?

F. Frankel, Massachusetts Institute of Technology, Cambridge: Envisioning science.

F. Frankel, M. Hucka, Y. Lazebnik, S. Wodak

SESSION 3: Biological Systems and Representations Thereof II
Chairperson: R. Brent, The Molecular Sciences Institute, Berkeley, California

H. Bolouri, Institute for Systems Biology, Seattle, Washington: Appropriate representations for modeling genes and genetic regulatory networks.
D.L. Cook, University of Washington, Seattle: BioD: Basis for an ontology of biological functions?
V. Schachter, Genoscope, Evry, France: Formal languages for core molecular biology.
P.F. Nielsen, University of Auckland, New Zealand: Ontologies for describing biological processes.
A. Gilman, Lawrence Berkeley National Laboratory, California: The Berkeley BioSPICE conceptual framework: Representing formal and informal knowledge with multiple degrees of detail.
A. Regev, Harvard University, Cambridge, Massachusetts: Life of Pi: Process algebras as calculi for biomolecular processes.

General Discussion: Limits of Current Representations?

SESSION 4: Integrated Biological Modeling Environments
Chairperson: H. Bolouri, Institute for Systems Biology, Seattle, Washington

T. Sakurada, Keio University, Tsuruoka, Japan: The E-Cell modeling environment.
J. Schaff, University of Connecticut Health Center, Farmington: The Virtual Cell project.
B. Mishra, Courant Institute, New York University, New York: Cell talk/Sympatica.
C. Shaffer, Virginia Tech, Blacksburg: User interface paradigms for describing pathway models.

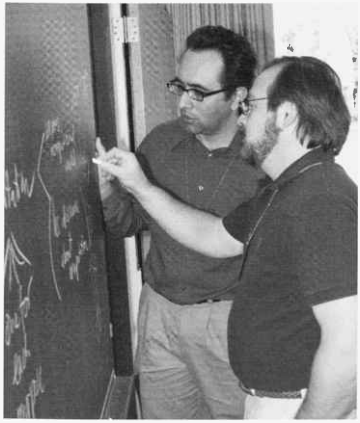
General Discussion: Future Requirements and Path Forward

SESSION 5: Languages Underlying Representation and Community Issues
Chairperson: A. Finney, University of Hertfordshire, Herts, United Kingdom

A. Finney, University of Hertfordshire, Herts, United Kingdom: Systems biology markup language: Level 2 and beyond.
J. Ambrosiano, Los Alamos National Laboratory, New Mexico: Rule-based models: Compact representation of large cellular networks.
J. Webb, BBN Technologies, Cambridge, Massachusetts: Language design considerations for composite models of biological systems.
J. Cassatt, National Institute of General Medical Sciences, Bethesda, Maryland: Mathematics, engineering, physics, and biology: Challenges for funding agencies.

General Discussion: Flourishing Orthography?

Closing Remarks:
D. Endy, Massachusetts Institute of Technology, Cambridge
A. Finney, University of Hertfordshire, Herts, United Kingdom
Y. Lazebnik, Cold Spring Harbor Laboratory



D. Chklovskii, J. Ambrosiano

Neural Circuits: Principles of Design and Operation

April 27–30

FUNDED BY **The Swartz Foundation**

ARRANGED BY **K.D. Miller,** University of California, San Francisco
H.S. Seung, Massachusetts Institute of Technology, Cambridge

SESSION 1: General Circuit Properties
Chairperson: D. Kleinfeld, University of California, San Diego

D. Chklovskii, Cold Spring Harbor Laboratory: (Potential) connectivity in cortical circuits.
S. Laughlin, University of Cambridge, United Kingdom: Stochastic limits to the design of energy-efficient neural circuits.

SESSION 2: General Cortical Properties I
Chairperson: J.A. Hirsch, University Southern California, Los Angeles

R. Yuste, Columbia University, New York: Imaging the structure and dynamics of the cortical microcircuit.
L.F. Abbott, Brandeis University, Waltham, Massachusetts: Controlling and supervising cortical circuits.
J.C. Hawkins, Redwood Neuroscience Institute, Menlo Park, California: General mechanisms of neocortical memory.
S. Makeig, University of California, San Diego: Are brain circuits multiscale?

SESSION 3: General Cortical Properties II
Chairperson: J.A. Hirsch, University of Southern California, Los Angeles

H. Markram, Brain & Mind Institute, Lausanne, Switzerland: Molecular basis of electrical diversity of interneurons.
G. Tamas, University of Szeged, Hungary: Sources and targets of slow inhibition in the neocortex.
A.M. Thomson, University of London School of Pharmacy, London, United Kingdom: Frequency and pattern filtering at cortical synapses.

SESSION 4: Visual Cortex I
Chairperson: L.F. Abbott, Brandeis University, Waltham, Massachusetts

A. Bell, Redwood Neuroscience Institute, Menlo Park, California: Nonlinear, multilayer ICA and the cortical visual processing hierarchy.
E.M. Callaway, The Salk Institute for Biological Studies, La Jolla, California: Functional organization of color-opponent input to primary visual cortex.
D.L. Ferster, Northwestern University, Evanston, Illinois: Testing models of visual cortical function.

A. Thomson, D. Chklovskii

SESSION 5: Visual Cortex II
Chairperson: L.F. Abbott, Brandeis University, Waltham, Massachusetts

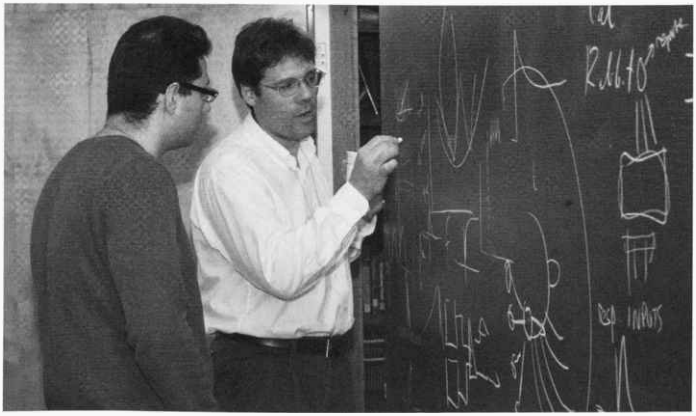
Y. Fregnac, Institut Alfred Fessard-CNRS, Gif sur Yvette, France: Shunting inhibition and computational diversity in visual cortex.
J.A. Hirsch, University of Southern California, Los Angeles: Inhibitory circuits at the first stage of visual cortical processing.
J.S. Lund, University of Utah, Salt Lake City: Real-life visual cortical circuits as substrates for its functional properties.
K.D. Miller, University of California, San Francisco: Role of dominant feedforward inhibition in cat V1 layer 4.
M. Shelley, New York University, New York: Coarse-graining of neuronal networks and the visual cortex.

SESSION 6: Nonvisual Cortical/Forebrain Systems
Chairperson: H. Markram, Brain & Mind Institute, Lausanne, Switzerland

D. Kleinfeld, University of California, San Diego: Attention, filtering, and mixing: Nonlinear coding blocks in the rat somatosensory motor system.
C. Brody, Cold Spring Harbor Laboratory: A computational model of the mammalian olfactory bulb.
J.J. Hopfield, Princeton University, New Jersey: Deriving "learning rules" for spike-timing based computation from the necessity of network self-repair.
M. Fee, Bell Laboratories, Murray Hill, New Jersey: Neural mechanisms of sequence generation in a songbird.
H.S. Seung, Massachusetts Institute of Technology, Cambridge: Learning by reinforcement of stochastic synaptic transmission in spiking neural network.
H.A. Swadlow, University of Connecticut, Storrs: A thalamocortical feedforward inhibitory network: Principles and operations in the awake state.
X.-J. Wang, Brandeis University, Waltham, Massachusetts: A basic cortical microcircuit model of working memory with three subclasses of inhibitory neurons.
A. Zador, Cold Spring Harbor Laboratory: Computation in auditory cortex.

SESSION 7: Silicon/Robotics
Chairperson: J.J. Hopfield, Princeton University, New Jersey

K.A. Boehen, University of Pennsylvania, Philadelphia: From local microcircuits to cortical maps.
K.K. Likharev, Stony Brook University, New York: CrossNets: Possible molecular electronics neural circuits.
S.-C. Liu, University and ETH Zurich, Switzerland: Spike-based vision system.
R. Sarpeshkar, Massachusetts Institute of Technology, Cambridge: Biologically inspired electronics.



C. Brody, M. Fee

Scientific Opportunities in Macromolecular Crystallography at NSLS-II

July 9–10

FUNDED BY **Brookhaven National Laboratory**

ARRANGED BY **W. Hendrickson,** Howard Hughes Medical Institute, Columbia University, New York
L. Joshua-Tor, Cold Spring Harbor Laboratory

Opening Remarks: **W. Hendrickson,** Howard Hughes Medical Institute, Columbia University, New York
L. Joshua-Tor, Cold Spring Harbor Laboratory

SESSION 1

S. Dierker, Light Sources Directorate, Brookhaven National Laboratory, Upton, New York: New storage ring design and characteristics.
L. Berman, National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York: Beamline optics and new detector developments.

SESSION 2

R. MacKinnon, Howard Hughes Medical Institute, The Rockefeller University, New York: Membrane proteins: State of the art, unique problems, and future needs.

SESSION 3

A. Joachimiak, Argonne National Laboratory, Illinois: Structural genomics.

Informal discussion: What Can the Structural Biologists Do to Further Support the Proposal?

SESSION 4

T. Steitz, Howard Hughes Medical Institute, Yale University, New Haven, Connecticut: Large complexes: Unique issues and future needs.
J.M. Hogle, Harvard University, Cambridge, Massachusetts: Virus structures.
P. Fitzgerald, Merck Research Laboratories, Rahway, New Jersey: Structure-assisted drug development.

SESSION 5: Summary and Next Steps

W. Hendrickson, Howard Hughes Medical Institute, Columbia University, New York
L. Joshua-Tor, Cold Spring Harbor Laboratory

W. Weiss, L. Joshua-Tor

W. Hendrickson, T. Steitz, H. Robinson

Taxonomy, DNA, and the Bar Code of Life

September 10–12

FUNDED BY **Alfred P. Sloan Foundation**

ARRANGED BY **J. Baker,** Academy of Natural Sciences, Philadelphia
J. Hanken, Harvard University, Cambridge, Massachusetts

Welcome and Introduction: J. Baker, Academy of Natural Sciences, Philadelphia, Pennsylvania
J. Hanken, Harvard University, Cambridge, Massachusetts

SESSION 1: DNA-based Species Identification: Conceptual Foundations
Chairperson: J. Hanken, Harvard University, Cambridge, Massachusetts

J.C. Venter, The Center for the Advancement of Genomics, Rockville, Maryland: Environmental shotgun sequencing.
R. DeSalle, American Museum of Natural History, New York
Recap of Banbury I.

Discussants
M. Stoeckle, The Rockefeller University, New York
J. Hanken, Harvard University, Cambridge, Massachusetts

P. Hebert, University of Guelph, Ontario, Canada
DNA-based species identification: State of the art.

Discussants
C. Moritz, University of California, Berkeley
G. Caccone, Yale University, New Haven, Connecticut
A. Bucklin, University of New Hampshire
S. Miller, Smithsonian Institution, Washington, D.C.
Are museum collections the appropriate place to begin a large-scale effort?

Discussants
R. Lane, The Natural History Museum, London, United Kingdom
G. Rosenberg, Academy of Natural Sciences, Philadelphia, Pennsylvania

SESSION 2: Implementing a DNA Bar-coding Initiative: Strategies, Specimens, Databases, Funding
Chairperson: J. Baker, Academy of Natural Sciences, Philadelphia, Pennsylvania

M. Stoeckle, The Rockefeller University, New York: Organizational strategies: Early action plan vs. museum consortium.

Discussants
P. Hastings, Scripps Institution of Oceanography, La Jolla, California
M. Graham, Canadian Museum of Nature, Ottawa, Ontario, Canada
G. Caccone, Yale University, New Haven, Connecticut
J.L. Edwards, Global Biodiversity Information Facility, Copenhagen, Denmark

R. Phelan, G. Caccone

Museum specimens: How many are there, and how are they preserved? Which types are most appropriate for a DNA bar-coding effort? Which ones should be avoided?

Discussants
C. Moritz, University of California, Berkeley
S.V. Edwards, University of Washington, Seattle
R. DeSalle, American Museum of Natural History, New York

Databases.

Discussants
J.L. Edwards, Global Biodiversity Information Facility, Copenhagen, Denmark
S. Federhen, National Library of Medicine, NIH, Bethesda, Maryland
P. Hebert, University of Guelph, Ontario, Canada
J. Baker, Academy of Natural Sciences, Philadelphia, Pennsylvania

Costs and funding. How much, and who will pay?

Discussants
J. Omura, The Presidio of San Francisco, California
R. Phelan, All Species Foundation, San Francisco, California

SESSION 3: Establishing Consensus Plans
Chairpersons: J. Baker, Academy of Natural Sciences, Philadelphia, Pennsylvania; **J. Hanken,** Harvard University, Cambridge, Massachusetts

- Is there life after Banbury II and, if so, what is it? What next?
- Working groups, initial funding strategies, statement of purpose.
- Statement of purpose.

J. Marchioni, D. Janzen, S. Miller, J. Edwards

Integrating Progress in the Genetics and Neuropharmacology of Schizophrenia

September 14–17

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **C.R. Cloninger,** Washington University Medical School, St. Louis, Missouri
J.T. Coyle, Harvard Medical School, Belmont, Massachusetts

SESSION 1: Overview of Current Status of the Molecular Genetics of Schizophrenia
Chairperson: C.R. Cloninger, Washington University Medical School, St. Louis, Missouri

C.R. Cloninger, Washington University Medical School, St. Louis, Missouri: General update and overview of the genetics of schizophrenia.
K. Stefansson, DeCode Genetics, Reykjavik, Iceland: Studies of neuregulin 1 by DeCode.
A. Corvin, St. James Hospital, Dublin, Ireland and D. Morris, St. James Hospital, Dublin, Ireland: Refinement of the "at risk" haplotype for schizophrenia.
I. Chumakov, Genset, Evry, France: Studies of G72 and DAAO in schizophrenia by Genset.
D.R. Weinberger, National Institute of Mental Health, NIH, Bethesda, Maryland: Critique of genetics of NMDA hypofunction in schizophrenia.

SESSION 2: Neural Systems Approaches to the NMDA Hypofunction Model of Schizophrenia
Chairperson: J.T. Coyle, Harvard Medical School, Belmont, Massachusetts

J.T. Coyle, Harvard Medical School, Belmont, Massachusetts: Overview of NMDA hypofunction model.
S. Grant, University of Edinburgh, United Kingdom: A core molecular mechanism for cognition and its disorders.
D. Javitt, Nathan Kline Institute for Psychiatric Research, Orangeburg, New York: Interactions of NMDA and dopamine systems and therapeutic targets.
J.H. Krystal, Yale University School of Medicine, West Haven, Connecticut: Interactions of NMDA and serotonin systems and therapeutic targets.
J.W. Newcomer and N.B. Farber, Washington University School of Medicine, St. Louis, Missouri: Developmental implications of NMDA receptor hypofunction.
C.A. Tamminga, University of Texas Southwestern Medical Center, Dallas: Critique of status of neuropharmacology of schizophrenia.

J. Coyle, D. Weinberger

J. Watson, R. Cloninger

SESSION 3: Other Genetic and Epigenetic Influences on Risk of Schizophrenia
Chairperson: M.T. Tsuang, Harvard Institute of Psychiatric Epidemiology & Genetics, Journal of Neuropsychiatric Genetics, Boston, Massachusetts

M. Owen, University of Wales College of Medicine, Cardiff, United Kingdom: Overview of nonglutamate genetic factors and their interactions influencing the risk of schizophrenia.
D. Blackwood, The Royal Edinburgh Hospital, United Kingdom: A chromosome 1q locus for schizophrenia.
S. Leonard, University of Colorado Health Sciences Center, Denver: α7 nicotinic receptor locus and NMDA interactions in schizophrenia.
R.E. Straub, National Institute of Mental Health, NIH, Bethesda, Maryland: Dysbindin (DTNBP1, 6p22.3): SNP detection, cognitive and fMRI phenotypes, and analysis of transcript and protein levels.

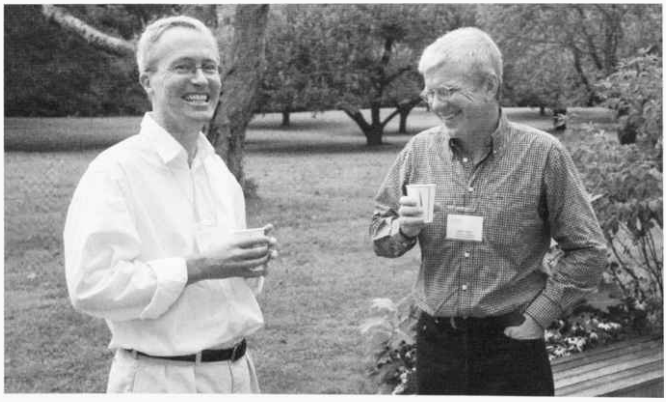
SESSION 4: Technological Opportunities for Accelerating Progress
Chairperson: J.W. Olney, Washington University Medical School, St. Louis, Missouri

M. Laruelle, New York State Psychiatric Institute, Columbia University, New York: Imaging NMDA-DA interactions in working memory and schizophrenia.
R. Schwarcz, Maryland Psychiatric Research Center, Baltimore: Studying NMDA-α7 nicotinic interactions with novel mGluR ligands.
S. Heckers, McLean Hospital, Belmont, Massachusetts: Regulation of hippocampal neurons in schizophrenia.
J. Eberwine, University of Pennsylvania Medical School, Philadelphia: Single cell chip technologies for genomics and proteomics.
M.A. Geyer, University of California, San Diego: Gluamatergic influences on sensorimotor gating in rodents.

SESSION 5: Where Do We Go from Here to Understand the Clinical Reality of Schizophrenia and Schizotypy?
Chairperson: E.M. Scolnick, Merck Research Laboratories, West Point, Pennsylvania

M.T. Tsuang, Harvard Institute of Psychiatric Epidemiology & Genetics, Journal of Neuropsychiatric Genetics, Boston, Massachusetts: A psychiatric geneticist's perspective.
W.T. Carpenter, University of Maryland, Baltimore: A psychopharmacologist's perspective.
Pablo Gejman, University of Chicago, Illinois: A molecular geneticist's perspective.

Panel Discussion:
K. Stefansson, DeCode Genetics, Reykjavik, Iceland
D.R. Weinberger, National Institute of Mental Health, NIH, Bethesda, Maryland
J.W. Olney, Washington University Medical School, St. Louis, Missouri
D. Goldman, National Institute on Alcohol Abuse and Alcoholism, NIH, Rockville, Maryland



S. Grant, M. Owen

Regulation of Inflorescence Morphology: Insights from Genetics and Genomics

September 21–24

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **E.A. Kellogg,** University of Missouri, St. Louis
D. Jackson, Cold Spring Harbor Laboratory

SESSION 1: Getting Started: Control of the Floral Transition
Chairperson: T. Rocheford, University of Illinois, Urbana

G. Coupland, Max-Planck-Institute for Plant Breeding Research, Koeln, Germany: Variation in the mechanisms that control flowering time.

T. Brutnell, Boyce Thompson Institute, Ithaca, New York: Role of phytochromes in the regulation of flowering time in maize.

D. Laurie, John Innes Centre, Norwich, United Kingdom: Genetic control of flowering time in barley and wheat.

M. Edgerton, Monsanto-Ceregen, St. Louis, Missouri: Alteration of flowering time in corn.

J. Colasanti, University of Guelph, Ontario, Canada: Leaf-derived floral inductive signals in maize.

SESSION 2: Development Mechanisms I: Inflorescence Structure and Function
Chairperson: S. Hake, U.S.D.A. Plant Gene Expression Center, Albany, California

E.A. Kellogg, University of Missouri, St. Louis: Grass flowers and inflorescences: New interpretations from comparative data.

K. Ikeda, University of Tokyo, Japan: Inflorescence and spikelet development in rice and functions of the *APO* gene.

D. Jackson, Cold Spring Harbor Laboratory: Fasciation and control of seed row number in maize.

W. Bruce, Pioneer Hi-Bred International, Inc., Johnston, Iowa: Characterizations of a maize *CLAVATA3* functional homolog.

SESSION 3: QTL and Association Mapping
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

E.S. Buckler, North Carolina State University, Raleigh: Using diverse maize germplasm to dissect inflorescence traits.
T. Rocheford, University of Illinois, Urbana: QTL approaches to study of maize inflorescence architecture.
D. Zamir, Hebrew University of Jerusalem, Rehovot, Israel: Real-time QTL of yield.

SESSION 4: Molecular Evolution
Chairperson: E. Vollbrecht, Cold Spring Harbor Laboratory

E. Kramer, Harvard University, Cambridge, Massachusetts: Two roads diverged in a wood: The elusive nature of genetic orthology.
M.D. Purugganan, North Carolina State University, Raleigh: Molecular evolution of *Arabidopsis* shoot architecture.
R.M. Clark, University of Wisconsin, Madison: The complexity of selection at a major effect QTL in maize, *teosinte branched 1* (*tb1*).
M. Frohlich, The Natural History Museum, London, United Kingdom: Suggestions for Evo-devo research.

SESSION 5: Developmental Evolution
Chairperson: E.A. Kellogg, University of Missouri, St. Louis

P. Soltis, University of Florida, Gainesville: Sequence analysis and expression patterns of floral genes in basal angiosperms.
E. Vollbrecht, Cold Spring Harbor Laboratory: *Ramosa1* and branching in the grass inflorescence.
D. Baum, University of Wisconsin, Madison: Role of meristem identity genes in the parallel evolution of inflorescence architecture in Brassicaceae.
W. Rottmann, ArborGen, Summerville, South Carolina: Response of sweetgum to overexpression of *LEAFY*.

SESSION 6: Genomics Approaches
Chairperson: V. Brendel, Iowa State University, Ames

R. Lucito, Cold Spring Harbor Laboratory: ROMA: Oligonucleotide arrays for the detection of gene copy number fluctuations.
V. Brendel, Iowa State University, Ames: Computational approaches to identify candidate genes involved in inflorescence development.
G. Chuck, USDA, ARS, Albany, California: Microarray analysis of the spikelet meristem identity mutants branched *silkless 1* and *frizzy panicle1* of maize and rice.
E.D. Brenner, New York University, New York: A genomic approach to study seed development in the basal gymnosperms: Cycads and ginkgo.

SESSION 7: Developmental Mechanisms II: Floral Organ Polarity, Number, and Size
Chairperson: D. Jackson, Cold Spring Harbor Laboratory

E.E. Irish, University of Iowa, Iowa City: Polarity of the maize flower and its imposition by the inflorescence.
H. Hirano, The University of Tokyo, Japan: Regulation of floral organ number and identity in rice.
J. Fletcher, USDA Plant Gene Expression Center, Albany, California: Role of *ULTRAPETALA* in regulating *Arabidopsis* shoot and floral meristem activity.
Y. Eshed, Weizmann Institute of Science, Rehovot, Israel: Regulation of plant organ size: A story of bread and butter.
S. Hake, USDA Plant Gene Expression Center, Albany, California: Role of meristem size on developmental fate.

M. Timmermans, H. Hirano

The Biology of Neuroendocrine Tumors

September 28–30

FUNDED BY **Verto Institute, LLC**

ARRANGED BY **A.J. Levine,** Institute for Advanced Studies, Princeton, New Jersey
E. Vosburgh, Verto Institute, LLC, Stamford, Connecticut

Introductory Remarks: **A.J. Levine,** Institute for Advanced Studies, Princeton, New Jersey: Verto Research Program Overview

SESSION 1: Neural Crest and Neuroendocrine Development
Chairperson: T. Jacks, Massachusetts Institute of Technology, Cambridge

J.A. Epstein, University of Pennsylvania, Philadelphia: Using mouse models to elucidate the developmental biology of the neural crest.

S.K. Kim, Stanford University School of Medicine, California: Signaling pathways regulating neuroendocrine cell differentiation and proliferation in the developing pancreas.

M.L. Meyerson, Harvard Medical School, Boston, Massachusetts: Functional studies of the multiple endocrine neoplasia type I (*MEN-1*) gene.

Keynote Speaker:
A.J. Levine, Institute for Advanced Studies, Princeton, New Jersey: Autophagy and cancer.

SESSION 2: Neuroendocrine Receptors
Chairperson: L.B. Chen, Dana-Farber Cancer Institute, Lexington, Massachusetts

G. Rindi, Universita degli Studi, Parma, Italy: VGF expression in the neuroendocrine system and related growths.

R. Salgia, University of Chicago, Illinois: Role of receptor kinases in neuroendocrine tumors.

R.V. Lloyd, Mayo Clinic, Rochester, Minnesota: EGFR in neuroendocrine (carcinoid) tumors.

V. Giandomenico, Uppsala University, Sweden: Identification of carcinoid-associated antigens for vaccine development.

D. Agrawal, H. Lee Moffitt Cancer Center and University of South Florida, Tampa: Development and utilization of carcinoid cell models for molecular analysis.

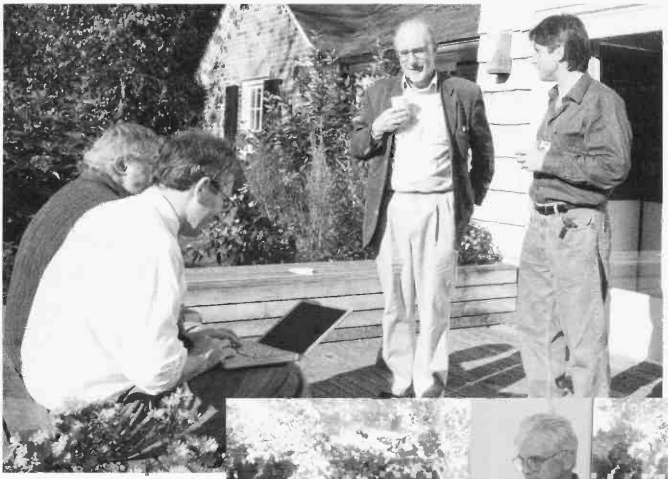
M. Meyerson, A. Levine, K. Peters

SESSION 3: Genetic Association Studies
Chairperson: J. Hoh, Yale University School of Medicine, New Haven, Connecticut

J. Ott, The Rockefeller University, New York: Statistical principles of human gene mapping.
C. Sing, University of Michigan Medical Center, Ann Arbor: Insights from genome association studies of common diseases.

SESSION 4: Clinical Update on Neuroendocrine Cancers
Chairperson: K. Oberg, University Hospital, Uppsala, Sweden

K. Oberg, University Hospital, Uppsala, Sweden: Position emission tomograph (PET) in the management of neuroendocrine tumors.
L. Kvols, H. Lee Moffitt Cancer Center and University of South Florida, Tampa: An update on radiolabled peptide therapy of carcinoid tumors.
J. Yao, Gastrointestinal Medical Oncology, Houston, Texas: Phase II study of Imatinib in patients with metastatic or unresectable carcinoid tumor.
M.H. Kulke, Dana-Farber Cancer Institute, Boston, Massachusetts: Clinical trials in neuroendocrine tumors: Role of molecularly targeted therapies.



Discussion on the deck

C. Harris, E. Vosburgh, S. Jin

Mouse Genome-wide Targeted Mutagenesis

September 30–October 1

FUNDED BY **Cold Spring Harbor Laboratory**

ARRANGED BY **R.P. Woychik,** The Jackson Laboratory, Bar Harbor, Maine
C.P. Austin, National Human Genome Research Institute, NIH

Welcome and Summary of Expectations: R.P. Woychik, The Jackson Laboratory, Bar Harbor, Maine

SESSION 1: Define What Type of Mutations Would be Most Desirable in a Genome-wide Collection

Review nature of mutations created by each gene-based mutagenesis technology: Debate pros, cons, and costs of each approach.

Moderator: G.M. Duyk, Exelixis Inc., South San Francisco, California

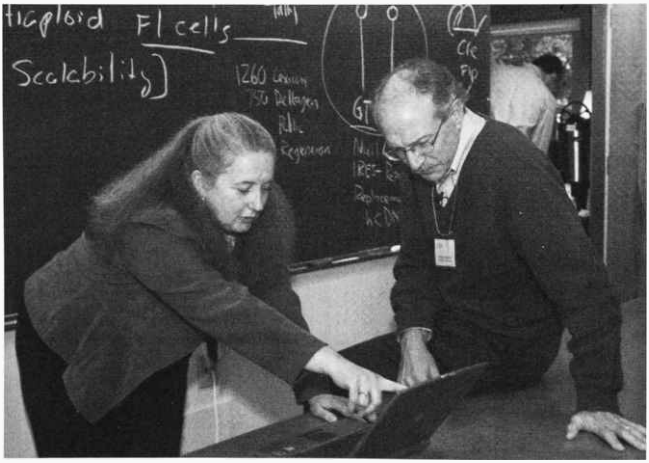
- Homologous recombination: M.R. Capecchi, University of Utah, Salt Lake City
- Gene-trapping approaches: B. Skarnes, Wellcome Trust Sanger Institute, Cambridge, United Kingdom, and B. Zambrowicz, Lexicon Genetics, The Woodlands, Texas
- RNAi: I.M. Verma, The Salk Institute for Biological Studies, La Jolla, California
- Transgenic approaches/universal integration sites: N. Heintz, The Rockefeller University, New York

SESSION 2: Desired Scope of the Project

Is there a compelling scientific case to undertake creating mutations in all genes in the genome?
To what extent do the currently available resources begin to address the needs of the biomedical research community?
Are current technologies and approaches efficient enough to meet the need, and what would be the time frame for completion?

Moderator: A. Bradley, The Wellcome Trust Sanger Institute, Cambridge, United Kingdom

- High-throughput KO technology: M.W. Moore, Deltagen, Inc., Redwood City, California
- Omnibank approach: A.T. Sands, Lexicon Genetics, The Woodlands, Texas
- Regeneron approach: G.D. Yancopoulos, Regeneron Pharmaceuticals, Inc. Tarrytown, New York
- Summary/Discussion



J. Eppig, R. Woychik

SESSION 3: Define Approaches to Cost Effectively and Efficiently Maintain a Targeted Mutant Mouse Resource

Which reagents (e.g., DNA targeting constructs, ES cells, and frozen embryos) would be available for distribution?
What is the cost of maintaining the resource?
To what extent should the mutant lines be phenotyped as part of a large-scale effort?

Moderator: T. Magnuson, University of North Carolina, Chapel Hill

- ES cells/chimeras/mice: A. Nagy, University of Toronto, Canada
- Cryopreservation (sperm/ICSI/Frozen embryos): K.C.K. Lloyd, University of California, Davis
- High-throughput phenotyping: M.W. Moore, Deltagen, Inc., Redwood City, California
- Summary/discussion

SESSION 4: Define the Nature of a Project Database, End-user Needs, Integrating Data into Current Resources, and Intellectual Property Issues
Moderator: J.T. Eppig, The Jackson Laboratory, Bar Harbor, Maine

- Database needs and integration resources: J.T. Eppig, The Jackson Laboratory, Bar Harbor, Maine
- IP and distribution issues: G.M. Duyk, Exelixis, Inc., San Fransicso, California
- Summary/discussion

SESSION 5: Open Discussion/Debate to Define the Scientific Nature of a Large-scale Consensus Project
Moderator: F.S. Collins, National Human Genome Research Institute, NIH, Bethesda, Maryland

We will start with a presentation of a "model project" based on discussions from the preceding sessions. Participants will be asked to modify the model during the course of the discussion to arrive at a final product with which to go forward.

- Nature of the mutations to be generated
- Scope of the project
- Nature of the resources to be distributed
- Informatics needs
- IP
- Cost and time line

SESSION 6: Wrap Up, Next Steps
Moderator: R.P. Woychik, The Jackson Laboratory, Bar Harbor, Maine

A. Roses, M. Moore

Feasible Solutions to Global Vaccine Shortages

October 8–10

FUNDED BY **Albert B. Sabin Vaccine Institute**

ARRANGED BY **L.A. Miller,** Intermedica, Inc., Darien, Connecticut
N.E. Tomich, U.S. Medicine Institute, Washington, D.C.

Welcome: H.R. Shepherd, Albert B. Sabin Vaccine Institute, New Canaan, Connecticut

SESSION 1:

Introductory Remarks: David Heymann, World Health Organization, Geneva, Switzerland: Polio surveillance and immunization program.

Charge to Conference, Conference Co-Chairs:
L.A. Miller, Intermedica, Inc., Darien, Connecticut and
N.E. Tomich, U.S. Medicine Institute, Washington, D.C.

Keynote Speaker: S. Cochi, National Immunization Program, CDC, Atlanta, Georgia

SESSION 2: Stockpiling
Chairpersons: E. O'Mara, Centers for Disease Control & Prevention, Atlanta, Georgia; **S. Bice,** Centers for Disease Control & Prevention, Atlanta, Georgia

Task Force Report

Task Force Panel:
S. Cochi, National Immunization Program, Centers for Disease Control & Prevention, Atlanta, Georgia
C.A. deQuadros, Albert B. Sabin Vaccine Institute, Washington, D.C.
D.C. Peterson, Immunization Action Coalition, St. Paul, Minnesota
E. Wilder, National Immunization Program, Centers for Disease Control & Prevention, Atlanta, Georgia

SESSION 3: Open Discussion and Consensus: Stockpiling
Moderators: L.A. Miller, Intermedica, Inc., Darien, Connecticut; **N.E. Tomich,** U.S. Medicine Institute, Washington, D.C.

SESSION 4: International Commission on Harmonization
Chairperson: J. Milstein, University of Maryland School of Medicine, Montpelier, France

Task Force Report

Task Force Panel:
A. Homma, Bio-Manguiinhos/Fiocruz, Rio de Janeiro, Brazil
S. Jadhav, Serum Institute of India, Pune
S.H. Lee, Center for Biologics Evaluation, Seoul, Korea
J.I. Santos, National Immunization Council, Juarez, Mexico

L. Cooper, L. Miller

SESSION 5: Open Discussion and Consensus: ICH
Moderators: L.A. Miller, Intermedica, Inc., Darien, Connecticut; **N.E. Tomich,** U.S. Medicine Institute, Washington, D.C.

SESSION 6: Public Advocacy
Chairperson: J.E. Fischer, Council on Foreign Relations, Washington, D.C.

Task Force Report

Task Force Panel:
L. Cooper, Columbia University College of Physicians & Surgeons, New York
S. Feldman, Aventis USA, Swiftwater, Pennsylvania
C.M. Grant, Aventis USA, Swiftwater, Pennsylvania
H. Larson, UNICEF, New York
R. MacDougall, Albert B. Sabin Vaccine Institute, Inc., Washington, D.C.
C. Ruppel, Every Child By Two, Washington, D.C.

SESSION 7: Open Discussion and Consensus: Public Advocacy
Moderators: L.A. Miller, Intermedica, Inc., Darien, Connecticut; **N.E. Tomich,** U.S. Medicine Institute, Washington, D.C.

R. Giffin, The National Academies, Washington, D.C.: Key elements of Institute of Medicine Report
SESSION 8: Financing
Chairperson: S. Jarrett, UNICEF, New York

Task Force Report

Task Force Panel:
D. Braga, Aventis USA, Swiftwater, Pennsylvania
A. Robbins, Tufts University School of Medicine, Boston, Massachusetts
J.C. Sadoff, Aeras Global TB Vaccine Foundation, Rockville, Maryland
D. Salisbury, Skipton House, London, United Kingdom
L. Tan, American Medical Association, Chicago, Illinois

SESSION 9: Open Discussion and Consensus: Financing

Where Do We Go From Here?
L.A. Miller, Intermedica, Inc., Darien, Connecticut
N.E. Tomich, U.S. Medical Institute, Washington, D.C.
Linking together the consensus statements
Prioritizing the action steps

Eugenics, Genes, and Human Behavior

October 14–16

FUNDED BY **National Human Genome Research Institute, NIH**

ARRANGED BY **D. Micklos,** Dolan DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
D. Micklos, Dolan DNA Learning Center, Cold Spring Harbor Laboratory

SESSION 1: History
Chairperson: S. Selden, University of Maryland, College Park

N. Gillham, Duke University, Durham, North Carolina: Sir Francis Galton and the foundations of eugenics.
E. Carlson, Stony Brook University, New York: Bad seed, corrupted germ plasm, prized pedigrees, and eugenic worth.

SESSION 2: Impacts
Chairperson: D. Micklos, Dolan DNA Learning Center, Cold Spring Harbor Laboratory

P.A. Lombardo, University of Virginia, Charlottesville: Immigration and sterilization in the United States.
G.E. Allen, Washington University, St. Louis: The international influence of the U.S. eugenics movement.

SESSION 3: Behavioral Genetics I
Chairperson: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

C. Gilliam, Columbia University, New York: Searching for genes involved in behavioral disorders.

General Discussion
Moderator: S. Selden, University of Maryland, College Park

SESSION 4: Resources: Dolan DNA Learning Center

D. Micklos and J.A. Witkowski, Cold Spring Harbor Laboratory: Introduction to the Image Archive on the American Eugenics Movement.

SESSION 5: Behavioral Genetics II
Chairperson: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

N. Segal, California State University, Fullerton: Twin studies and heritability of behavioral traits.
K.R. Jamison, Johns Hopkins School of Medicine, Baltimore: Personal experiences with mental illness and eugenics.

General Discussion
Moderator: S. Selden, University of Maryland, College Park

N. Gillham, S. Selden

Participants in a discussion

Controlling the Differentiation of Pluripotent Cells

October 19–22

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

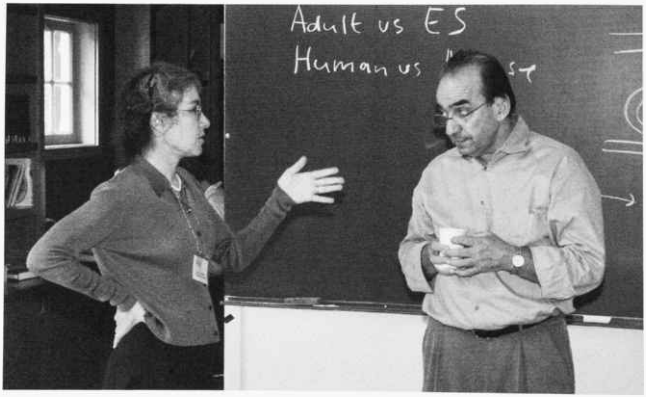
ARRANGED BY **R. McKay,** National Institute of Neurological Disorders and Stroke, NIH

SESSION 1: Controlling ES Cell Differentiation
Chairperson: N. Benvenisty, The Hebrew University of Jerusalem, Israel

N. Benvenisty, The Hebrew University of Jerusalem, Israel: Differentiation and genetic manipulation of human embryonic stem cells.
A. Smith, University of Edinburgh, United Kingdom: Pluripotency and differentiation of embryonic stem cells.
L. Studer, Memorial Sloan-Kettering Cancer Center, New York: Human ES cell in neural development and repair.
H. Niwa, RIKEN, Hyogo, Japan: Transcriptional regulation for self-renewal and differentiation of ES cells.
H.R. Scholer, University of Pennsylvania, Kennett Square: Derivation of germ cells from mouse embryonic stem cells.

SESSION 2: Differentiation of Mes Endoderm
Chairperson: G. Keller, Mount Sinai School of Medicine, New York

K. Zaret, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Specification of liver and pancreas progenitors from the embryonic endoderm.
H. Semb, Gothenburg University, Goteborg, Germany: Differentiation of human ES cells toward endoderm and pancreatic cell lineages.
C. Mummery, Netherlands Institute for Developmental Biology, Utrecht: Cardiomyocyte differentiation of human ES cells.
R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Reprogramming the genome.
A. Wobus, Institute of Plant Genetics Gatersleben, Germany: ES cell differentiation into functional beta-like cells and the role of nestin expression.
G. Keller, Mount Sinai School of Medicine, New York: Lineage induction and specification during embryonic stem cell differentiation.
R. McKay, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: Deriving the cellular phenotype.



L. Zoloth, R. McKay

SESSION 3: Stem Cells in the Life Cycle
Chairperson: R.A. Pedersen, Addenbrooke's Hospital, Cambridge, United Kingdom

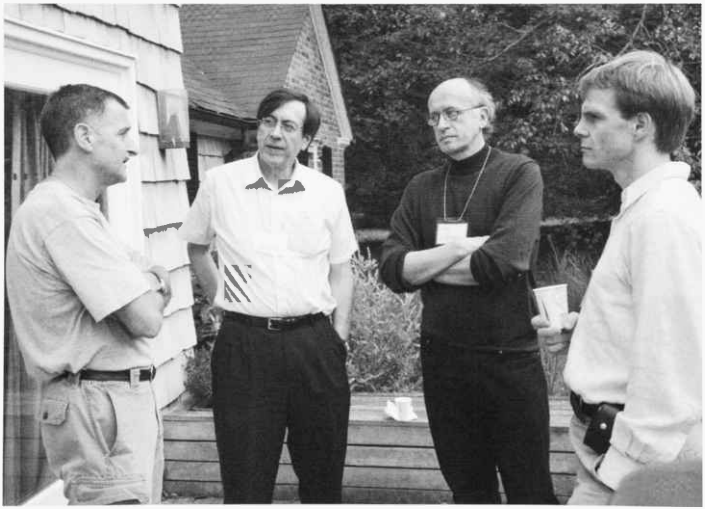
K. Hochedlinger, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Reprogramming of cancer cells by nuclear transfer.
P. Lanscorp, British Columbia Cancer Research Center, Vancouver: Telomerase and "self-renewal" of stem cells.

SESSION 4: What Is Ethically Possible?
Chairperson: R. McKay, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland

L.S. Zoloth, Northwestern University, Chicago, Illinois: Ethical considerations in the organization of research in pluripotent cells.

SESSION 5: Implementing the Promise of Pluripotent Cells from Mouse to Man
Chairperson: R. McKay, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland

R.R. Behringer, M.D. Anderson Cancer Center, Houston, Texas: Genetic studies of early mouse development.
R.A. Pedersen, Addenbrooke's Hospital, Cambridge, United Kingdom: Taking human ES cells to the clinic.
H. Weber, Medical Research Council, London, United Kingdom: The United Kingdom Stem Cell Initiative.
J.F. Battey, National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, Maryland: NIH support for human embryonic stem cell research.



J. Battey, R. Pedersen, G. Keller, L. Studer

Finding New Genes Linked to ALS: A Focus on Current Technologies and Their Potential Application

October 26–28

FUNDED BY **The ALS Association**

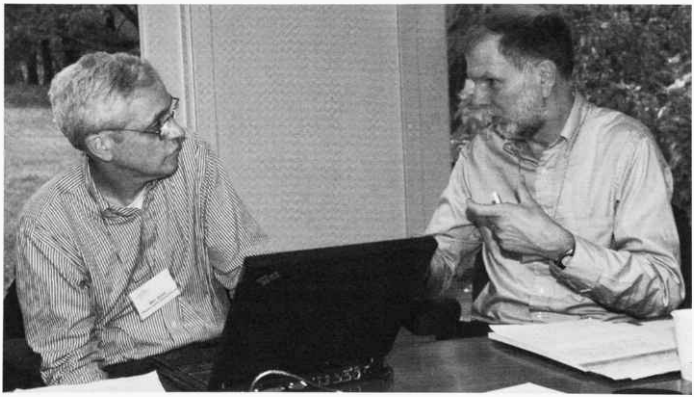
ARRANGED BY **R.H. Brown,** Massachusetts General Hospital, Charlestown
L. Bruijn, The ALS Association, Guilford, Connecticut

SESSION 1: Opening Remarks
Chairperson: L. Bruijn, The ALS Association, Guilford, Connecticut

R.V. Abendroth, The ALS Association, Milwaukee, Wisconsin: Opening remarks.
R.H. Brown, Massachusetts General Hospital, Charlestown: Introduction to ALS and ALS genetics.
D. Altshuler, Massachusetts General Hospital, Boston: The challenge of complex genetics: Overview.

SESSION 2: Statistical Approaches to Complex Genetics
Chairperson: A. Al Chalabi, Institute of Psychiatry, London, United Kingdom

E. Martin, Duke University Medical Center, Durham, North Carolina: Theoretical considerations: TDT, case control, other models.
D.B. Goldstein, University College London, United Kingdom: Approaches to haplotype reduction.
J. Haines, Vanderbilt University Medical Center, Nashville, Tennessee: Practical considerations: Size and power calculations.



R. Brown, K. Fischbeck

SESSION 3: Techniques
Chairperson: R. Baughman, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland

D.R. Cox, Perlegen Sciences, Inc. Mountain View, California: Chip-based SNP analysis.
P.J. de Jong, Children's Hospital Oakland Research Institute, California: Cloning approaches toward the sequencing of disease haplotypes.

SESSION 4: Precedents in Complex Analyses of Human Diseases
Chairperson: J. Haines, Vanderbilt University Medical Center, Nashville, Tennessee

S. Hauser, University of California, San Francisco: Investigations of selected neurological disorders.
D. Goldman, National Institute on Alcohol Abuse & Alcoholism, Rockville, Maryland: Genetic and functional analyses of serotonin transporter in affective disorders.

SESSION 5: Application of Complex Genetics to ALS
Chairpersons: R.H. Brown, Massachusetts General Hospital, Charlestown, and **P.J. de Jong,** Children's Hospital Oakland Research Institute, California

L. Bruijn, The ALS Association, Guilford, Connecticut: The challenge.
A. Al Chalabi, Institute of Psychiatry, London, United Kingdom: Complex genetics in ALS: Studies to date.
G.A. Rouleau, Montreal General Hospital, Canada: How do we proceed?

Discussion
Moderators: K.H. Fischbeck, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland, and **G.A. Rouleau,** Montreal General Hospital, Canada

D. Altshuler, D. Cox

Molecular Differentiation of Benign and Malignant Pheochromocytomas and Neuroblastomas

November 16–18

FUNDED BY **National Hypertension Association, Inc.**

ARRANGED BY **G. Eisenhofer,** National Institute of Neurological Disorders & Stroke, NIH, Bethesda, Maryland
W.M. Manger, National Hypertension Association, New York

Introductory Remarks and Summary of Expectations:
W.M. Manger, National Hypertension Association, New York

SESSION 1: Current Status of Diagnosis, Localization, Clinical Management, and Treatment
Chairperson: W.M. Manger, National Hypertension Association, New York

D.S. Goldstein, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: Diagnostic markers of malignancy in pheochromocytoma: Issues for biochemical and imaging approaches.

W.F. Young, Mayo Clinic, Rochester, Minnesota: Management of malignant pheochromocytoma.

N.-K.V. Cheung, Memorial Sloan-Kettering Cancer Center, New York: Neuroblastoma: A clinical enigma.

B.L. Shulkin, University Hospital, Ann Arbor, Michigan: 131I-MIBG therapy and new radiopharmaceuticals for pheochromocytoma.

SESSION 2: Tumor Genetics
Chairperson: H. Lehnert, University of Magdeburg, Germany

D. Smith, Mayo Clinic, Rochester, Minnesota: Microarrays and advanced technologies for the study of cancer.

H.P.H. Neumann, Medizinische Universitatsklinik, Freiburg, Germany: Malignant pheochromocytoma: Role of genetic screening.

P. Dahia, Dana-Farber Cancer Institute, Boston, Massachusetts: The growing complexity of the genetics of pheochromocytoma.

J.M. Maris, Children's Hospital of Philadelphia, Pennsylvania: Genetic basis for neuroblastoma heterogeneity.

G. Eisenhofer, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: Distinct gene expression profiles in hereditary and sporadic pheochromocytoma.

SESSION 3: Malignant Pheochromocytoma
Chairperson: H.P.H. Neumann, Medizinische Universitatsklinik, Freiburg, Germany

T.J. Giordano, University of Michigan Health System, Ann Arbor: Distinct transcriptional profiles of metastasizing and nonmetastasizing adrenal and extra-adrenal paragangliomas uncovered by DNA microarray analysis.

H. Lehnert, University of Magdeburg, Germany: Expression patterns of the telomeric complex and somatostatin receptor subtypes in benign and malignant pheochromocytoma: Implications for diagnosis and treatment.

R.R. de Krijger, Erasmus MC, Rotterdam, The Netherlands: Candidate gene and global genetic approaches to malignancy in pheochromocytoma.

K. Pacak, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: Proteomics, microarrays, and model systems for improved diagnosis and treatment of malignant pheochromocytoma.

SESSION 4: Models, Pathways, and Mechanisms of Tumorigenesis
Chairperson: S. Bornstein, University of Dusseldorf, Germany

A. Tischler, New England Medical Center, Boston, Massachusetts: Experimental models of pheochromocytoma.

L.A. Greene, Columbia University, New York: Regulation of pheochromocytoma differentiation and proliferation by ATF5.

A. Iavarone, Columbia University, New York: The N-*myc*–ID2-Rb pathway in neuroblastoma.

D. Yamashiro, Columbia University, New York: Angiogenic factors in neuroblastoma.

SESSION 5: Review of Presentations
Chairpersons: G. Eisenhofer, National Institute of Neurological Disorders & Stroke, NIH, Bethesda, Maryland; **W.M. Manger,** National Hypertension Association, New York

R. Lucito, Cold Spring Harbor Laboratory: High-resolution gene-copy-number detection using ROMA, representational oligonucleotide microarray analysis.

SESSION 6: Open Discussion Session
Moderator: S. Bornstein, University of Dusseldorf, Germany

Topic 1: Prognostic Markers and Targets for Treatment
H. Lehnert, University of Magdeburg, Germany
A. Tischler, New England Medical Center, Boston, Massachusetts
Topic 2: Gene Expression Profiling and Proteomics
D. Smith, Mayo Clinic, Rochester, Minnesota
Topic 3: Tissue Banks and Tissue Sharing
T.J. Giordano, University of Michigan Health System, Ann Arbor
P. Dahia, Dana-Farber Cancer Institute, Boston Massachusetts
Topic 4: Tissue Procurement and Data Management
F. Brouwers, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland
H.P.H. Neumann, Medizinische Universitat-sklinik, Freiburg, Germany
Topic 5: Models
A. Tischler, New England Medical Center, Boston, Massachusetts
Topic 6: Pheochromocytoma Research Consortium
K. Pacak, National Institute of Neurological Disorders & Stroke, NIH, Bethesda, Maryland
Topic 7: Funding, Future Interactions, and Directions
W.M. Manger, National Hypertension Association, New York
G. Eisenhofer, National Institute of Neurological Disorders & Stroke, NIH, Bethesda, Maryland

G. Eisenhofer, R. Lucito, W. Manger

Neural Representation and Processing of Temporal Patterns

December 14–17

FUNDED BY **Redwood Neuroscience Institute**

ARRANGED BY **C. Brody,** Cold Spring Harbor Laboratory
D. Buonomano, University of California, Los Angeles
J.C. Hawkins, Redwood Neuroscience Institute, Menlo Park, California

SESSION 1

J.C. Hawkins, Redwood Neuroscience Institute, Menlo Park, California: Temporal processing: Questions and definitions.
M.N. Shadlen, Howard Hughes Medical Institute, University of Washington, Seattle: Representation of elapsed time and hazard rates in parietal cortex of the macaque.
P.A. Tallal, Rutgers University, Newark, New Jersey: Neural signature of rapid auditory processing disorders in dyslexia: Insights from fMRI and remediation studies.
C. Brody, Cold Spring Harbor Laboratory: Timing and the neural representation of short-term memories.
X.-J. Wang, Brandeis University, Waltham, Massachusetts: A biophysical model of robust scalar timing.

SESSION 2

D. Buonomano, University of California, Los Angeles: Psychophysical and theoretical studies of simple and complex temporal processing.
M.P. Kilgard, University of Texas, Dallas: Temporal coding and plasticity in auditory cortex.
J.J. Hopfield, Princeton University, New Jersey: Encoding/recognizing short dynamic trajectories: Audition.
E. Ahissar, The Weizmann Institute, Rehovot, Israel: Temporal encoding and decoding in vibrissal active touch.
J.C. Hawkins, Redwood Neuroscience Institute, Menlo Park, California: Time, sequences, and movement in perception.

M. Kilgard, P. Tallal

SESSION 3

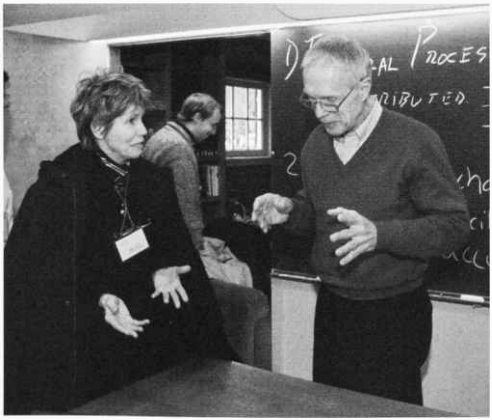
R. Ivry, University of California, Berkeley: The cerebellum and event timing.
M.D. Mauk, University of Texas, Houston Medical School: Timing in the cerebellum.
M. Fee, Bell Laboratories, Murray Hill, New Jersey: Neural representation of time in the songbird.
W.H. Meck, Duke University, Durham, North Carolina: Coincidence-detection models of interval timing.
R. Gallistel, University of California, Los Angeles: The temporal information content of a protocol as a determinant of acquisition latency.

SESSION 4

S. Hooper, Ohio University, Athens: Temporal processing by single neurons.
P. Verschure, Institute of Neuroinformatics, Zurich, Switzerland: Decoding information in a temporal pattern code.
R. Granger, University of California, Irvine: Derivation and analysis of sequential processing algorithms in thalamocortical circuitry.
T. Rammsayer, University of Goettingen, Germany: Neuropharmacological dissociation of distinct timing mechanisms in the brain.
D.L. Harrington, Veterans Affairs Medical Center, Albuquerque, New Mexico: Neural representation of interval encoding and decision making.

SESSION 5

D. Levitin, McGill University, Montreal, Canada: Temporal coherence in music and the prefrontal cortex.
M.R. Jones, Ohio State University, Columbus: The dynamics of attending to auditory events.
B.H. Repp, Haskins Laboratories, New Haven, Connecticut: Accentuation and coordination.
A. Kepecs, Cold Spring Harbor Laboratory: A model of dynamics of graded persistent activity.



M. Jones, J. Hopfield


DOLAN DNA
LEARNING CENTER
Bacteria
Divide
In Half

DOLAN DNA LEARNING CENTER

Preparing students and families to thrive in the gene age

ADMINISTRATION	INSTRUCTION	BIOMEDIA	TECHNOLOGY DEVELOPMENT
Judy Cumella-Korabik	**Elna Carrasco**	**Shirley Chan**	**Jennifer Aizenman**
Nancy Daidola	**Kimberly Kessler**	**Darius Farraye**	**Adrian Arva**
Mary Lamont	**Erin Maroney**	**Eun-Sook Jeong**	**Scott Bronson**
David Micklos	**Amanda McBrien**	**Susan Lauter**	**Uwe Hilgert**
Carolyn Reid	**Michael O'Brien**	**Bronwyn Terrill**	
Erin Wahlgren	**Danielle Sixsmith**	**Chun-hua Yang**	

The sequencing of the human genome, and the genomes of other model organisms, largely has been justified on predicted benefits to human health and agricultural productivity. Beyond these practical objectives is the larger goal of unifying diverse biological disciplines at the molecular level. In the genome age, biologists of all persuasions must learn to grapple with large-scale data sets—and become adept at moving between biological sequence, structure, function, and systematics.

Evolution has been called the only organizing theory in biology, and the National Science Education Standards specifically acknowledge that molecular and evolutionary biology are among the "small number of general principles that can serve as the basis for teachers and students to develop further understanding of biology." Now, the new discipline of comparative genomics offers a natural bridge between molecular and evolutionary biology. By working first-hand with genome data, students can gain a deeper understanding of evolutionary processes, the use of model systems in biology, and the close genetic relationships that exist between seemingly diverse organisms. Students may be rewarded with new and sometimes surprising insights into species relatedness, such as the recent demonstration that humans share a significant number of genes with corals!

Genomic sequences from hundreds of organisms are available to anybody with an Internet connection—as are bioinformatics tools that allow one to explore sequence data, predict the presence of genes, and compare features shared between different organisms. These freely available resources hold out the great promise of making modern biology an egalitarian pursuit open to virtually any biologist or student with a question to ask. Indeed, for the first time in the history of biology, novices can work with the same information, at the same time, and with the same tools as research scientists.

Although biological sequences and bioinformatics tools may be free, there are significant barriers to their widespread use by bioinformatics novices, including mid-career researchers, junior faculty, and students. There is a bewildering array of tools for analyzing sequence information, each with particular strengths and weaknesses. The larger "portal" sites may host a dozen or more tools and databases. The "nested" construction of these Internet sites often means that a particular tool or function is buried under several layers of organization. Effective use of many tools also requires the user to keep track of, and move between, several open browser windows. Most database and tool sites are starkly utilitarian, making few concessions to design or ease of use. Thus, one of the greatest challenges of the genome era is to get sequence data and tools into the hands of a broader base of biologists and to directly involve students in genome bioinformatics. This will require the creation of more intuitive, visually pleasing computer tools that engage novices and allow them to quickly learn the rudiments of genome browsing and sequence analysis.

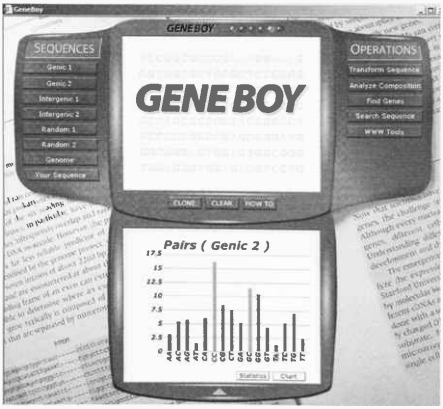
Flash Tools for Genome Bioinformatics

The DNALC began to rise to the challenge of bioinformatics in the mid 1990s, when we developed the first polymerase chain reaction (PCR)-based experiments to allow students to analyze their own DNA differences (polymorphisms) using simple agarose gel systems. This led to the development of the *Student Allele Database*, which allowed students to enter their class polymorphism data and compare it with research data from world populations. By the late 1990s, we had developed a simple system for amplifying mitochondrial (mt) DNA, and began to sequence DNA samples submitted from students around the country.

Our current *Bioservers* site (www.bioservers.org), released in 1999, introduced separate tools for analyzing allele data and sequence data in human populations. These intuitive interfaces allow students to enter their own data and draw data from a number of research sources, and then to manipulate the data sets in a common workspace. The *Allele Server* stores results of student experiments with the *Alu* insertion polymorphism PV92, and provides graphical tools for testing Hardy-Weinberg equilibrium and comparing allele and genotype frequencies. *Sequence Server* stores student mitochondrial control region sequences and provides tools for CLUSTALW analysis and BLAST searches. Registered users can save files of interest and return to their workspace to continue with projects they have initiated. Each of these tools conforms to several simple rules for constructing intuitive Internet tools: consistent and logical placement of tool bars, minimum use of submenus or "nested" screens, and minimal text.

In 2003, we began to experiment with "wrapping" sophisticated algorithms in an attractive multimedia "skin." In striving to create a simple and engaging interface, we finally gave in to our own analogy to a computer game. The result was *Gene Boy* (www.dnai.org/geneboy), a program with what we call "thingness"—the sense of being a real, onscreen object. One draws sequence data from buttons on the left side of the console and performs bioinformatic functions with buttons on the right: transform/format DNA sequence, analyze nucleotide composition, search for restriction sites or other short motifs, and find open reading frames. A "clone screen" feature allows the user to duplicate a result in a new screen for comparison with a second result, while "skin" color can be changed in the manner of desktop wallpaper.

Working with The Mill—a London post-production house that won an Academy Award for special effects in *Gladiator* and produced animated sequences for *Harry Potter I* and *II*—we also developed a



new way to visualize genome data. We color-coded all of the features of a 650,000-nucleotide region of human chromosome 11—gene exons and introns, as well as four classes of transposons ("jumping genes") and repeated DNA. We then created a three-dimensional (3-D) "perspective" view in which each feature of 100 nucleotides or longer is rendered as a colored key in a DNA "keyboard." The result is a narrated tour (www.dnalc.org/chr11/) that includes gene clusters encoding blood components (globins), transcription factors, and olfactory receptors. The 3-D tour makes it sensationally apparent that genes and intergenic regions are chock full of transposons. Because repetitive DNA is typically "masked" from genome presentations, this view is striking even for those who know that transposons account for about 50% of the human genome. Visualizing the extent of transposon intrusion on our genome makes it difficult not to be moved to ask the question: "Why?"

The development of *Gene Boy* and the *Chromosome 11 Tour* was made possible by the tight integration of design, programming, and biological knowledge in our Biomedia Group. We want to build upon these design prototypes to develop a set of full-functioned *Flash Tools* that provide new ways to visualize, manipulate, and compare genome data. The term *Flash Tools* embodies the fact that interfaces are built using the new-generation multimedia design software Flash MX, as well as their speed and ease of use. We envision a sequence analyzer with the ability to "decorate" a DNA sequence with features from successive analyses, as well as a 3-D genome browser that will literally add a new dimension to large-scale gene analysis. The interface will integrate with standard algorithms and reusable software components of the Generic Genome Browser at the Generic Model Organism Database (GMOD), which is co-managed by CSHL scientist Lincoln Stein.

HHMI Bioinformatics

In 2003, we concluded a four-year project, funded by the Howard Hughes Medical Institute (HHMI), to introduce students and teachers to principles of genome analysis. At the heart of the program is *VectorNet*, a portable computer laboratory consisting of 12 laptops linked to a laptop server via a wireless local area network (LAN). The server can distribute live Internet access through a single fast connection, or it can rely on its own mirror of the DNALC Internet site and local copies of bioinformatics tools and GenBank data sets. Fitting entirely in two plastic trunks, *VectorNet* converts any classroom or lecture space into a fully functional computer lab in minutes.

In the project's local program, *New York City Genes*, we worked intensively with 1965 students and 316 teachers from public high schools representing each of the five boroughs of New York City. In the national program, the *Vector Bioinformatics Workshop*, we provided training for 320 educators in 16 different locations:

Blood Center of Southeastern Wisconsin, Milwaukee, WI
Contra Costa County Office of Education, Pleasant Hill, CA
Coriell Institute for Medical Research, Camden, NJ
CIIT Center for Health Research, Research Triangle Park, NC
Fred Hutchinson Cancer Research Center, Seattle, WA
Foundation for Blood Research, Scarborough, ME
Kaiser Permanente Center for Health Research, Portland, OR
National Center for Biotechnology Information, Bethesda, MD
Oklahoma Medical Research Foundation, Okalahoma City, OK
Rockefeller University, New York, NY
Salk Institute for Biological Studies, La Jolla, CA
Southwest Foundation for Biomedical Research, San Antonio, TX
Stowers Institute for Medical Research, Kansas City, MO
Trudeau Institute, Saranac Lake, NY
Harbor-UCLA Research and Education Institute, Torrance, CA
Whitehead Institute for Biomedical Research, Cambridge, MA

During the teacher workshop, participants made extensive use of computer tools to learn principles of gene analysis including DNA sequence annotation, gene structure and regulatory elements, gene families and whole-gene analysis, functional genomics and DNA arrays, and gene discovery using single nucleotide polymorphisms (SNPs) and other markers. Participants also amplified two DNA polymorphisms and used their own data as a starting point to investigate DNA data sets, population genetics, human origins, and disease mechanisms—illustrating the crossover between DNA experiments done in vitro (test tubes) and in silico (computers).

Our experience with this course has shown that bioinformatics analysis is more difficult to teach than are the seemingly complex lab procedures of molecular genetic analysis. Experience with the CSHL

postgraduate *Genome Access* course confirmed that novice postgraduates face the same difficulties in utilizing Internet resources as do the innovative teaching faculty who participated in the HHMI course. Thus, DNALC staff member Uwe Hilgert was invited to become a co-instructor of the postgraduate *Genome Access* course—to integrate many of the instructional methods we developed for the HHMI bioinformatics course.

To gauge the impact of the *Vector Bioinformatics Workshop* on course participants, we conducted a follow-up survey of our 2001 alumni. Most participants reported that the workshop had enabled them to integrate into their own teaching instruction on the Human Genome Project (94.7%), bioinformatics (80%), DNA-typing/polymorphisms (78.2%), gene organization (76.4%), and human origins, evolution, and migration (80%). A significant number had integrated workshop content in hands-on computer exercises with their students (85.5%), including analysis of DNA sequence composition (73.3%), sequence searches (60%), sequence alignments (51.5%), construction of phylogenetic trees (46.7%), and gene prediction and/or protein structure examination (26.7%). A majority of respondents had also disseminated information about genomic biology/bioinformatics among colleagues, local teachers, and at workshops or meetings (73%).

Bioinformatics Lab Field Trips and Sequencing Service

The octagonal Bioinformatics Laboratory is the symbolic heart of our facility and is emblematic of the central role of computation in modern biology. A visit to the Bioinformatics Lab has been a key element of field trips for students doing PCR amplification of their own DNA polymorphisms. Middle school students have also used the laboratory for multimedia explorations of the forensic and DNA evidence used to identify the remains of the Romanov family (the last ruling family of Tsarist Russia), and to test whether or not claimant Anna Anderson was Anastasia Romanov. In 2003, we first offered a menu of pure bioinformatics field trips targeted at high school students:

- *Gene Sleuthing* Discover gene features that allow one to "mine" genes from the genomes of humans and other organisms.
- *Of Maize and Men: Jumping Genes Across Kingdoms* Uncover transposons in the genomes of corn and humans, discover how they "jump," and how they can contribute to disease.
- *Gleevec™: A Smart Bomb in the War on Cancer* Discover how the rational development of anti-cancer drugs critically depends on a thorough understanding of cell signaling pathways.
- *HIV: The Co-Evolution of Virus and Man* Discover how HIV evolved, how specific mutations lead to viral drug resistance, and why some people have natural resistance to infection.
- *Sickle Cell Anemia: A Disease of Diverse Populations* Discover the evolution of sickle cell disease, how the mutation affects red blood cells, and why the mutation persists in human populations.
- *Human Origins and the Story of Mitochondrial Eve* Reconstruct the human family tree to track down our most recent common maternal ancestor, and to discover where and when she lived.

During the year, our *Sequencing Service* processed more than 4000 student mitochondrial DNA samples submitted by 98 high schools and 56 universities and colleges. These sequences were then placed on our *Sequence Server*, where they can be viewed

Michael O'Brien leads a polymerase chain reaction (PCR) laboratory.

and analyzed by the students. The gift of a 377 DNA sequencer in 2000 by Applied Biosystems, plus continued donation of technical support and reagents, has allowed us to offer this service free-of-charge. However, demand is so great that this year we partnered with Dick McCombie's group at the Lita Annenberg Hazen Genome Sequencing Center to give us access to a fast, 96-capillary instrument. We are thankful for technical assistance from a number of Genome Center staff: Theresa Zutavern, Beth Miller, Andrew O'Shaughnessy, Lori Grady, and Jody Barditch.

New NSF Grants Increase Collaboration with CSHL Staff

Since its inception, the DNALC has looked to the research at CSHL for inspiration and a window on modern biology. On one hand, we attempt to predict which new areas of research may be important for students to learn about in the future. On the other hand, we try to identify "iconographic" lab experiments that illustrate new methods and subsume important biological principles. For example, our friendship with Doug Hanahan, in the mid 1980s, led us to adapt one of his simple methods to put DNA into bacteria. Today, this method is commonly used by high school and college students around the United States and Europe. The technique is so simple that we have been using it with 5th and 6th graders for several years.

Recently, we have been strengthening our ties to the main Laboratory campus, to the extent of submitting joint grants with CSHL researchers. The effort resulted in four awards from the National Science Foundation (NSF) on plant genetics and genomics, which represent a new degree of integration between high-level science and education outreach.

Plant molecular genetic and genomic research still lags behind medically oriented research on microbes and higher animals. As a result, there are relatively few lab experiences that expose college-level students to the growing insights into plants offered by genomic biology. Thus, our proposal to NSF to develop a laboratory- and Internet-based curriculum to bring college students up-to-the-minute with modern plant research struck a common chord with the six reviewers—all of whom rated it as "excellent." This is the first time in 15 years that any proposal we have submitted has received such a unanimous vote of confidence from NSF reviewers.

During the first year of this project, we developed a set of laboratories illustrating key concepts of gene analysis in plants, including the relationship between phenotype and molecular genotype, genetic modification of plants and detection of transgenes in foods, and linkage and bioinformatics methods for gene mapping. Key to the project are lab and bioinformatics exercises that will give students the unique opportunity to work with CSHL researcher David Jackson in determining the cellular analysis of *Arabidopsis* genes of unknown function. An Internet "super site," *Greenomes*, supports the laboratories with online protocols, custom analysis tools, shared databases, and collaborative bulletin boards. The new materials were introduced to Advisory Panel members at a workshop held June 16–20. Feedback from the Advisory Panel workshop is being used to refine curricula prior to initiating national dissemination workshops in 2004–2005. Faculty members from a number of key institutions attended the workshop:

Linnea Fletcher and Margaret Maher, Austin Community College
Robert Ballard and Barbara Speziale, Clemson University
Theresa Fulton and Sharon Mitchell, Cornell University
Erin Dolan, Fralin Biotechnology Center, Virginia Tech
Ed Himelblau, Long Island University at Southampton
Elizabeth Rosen and Noreen Warren, Madison Area Technical College
Katy Korsmeyer, Santa Clara Biotechnology Education Partnership
Leonore Reiser, The *Arabidopsis* Information Resource
Christine Pfund and Federico Luy, University of Wisconsin-Madison
David Jackson and Catherine Kidner, Lincoln Stein, CSHL

Later in the year, we received the news that the DNALC was co-awardee on three NSF research grants to sequence and annotate plant genomes. Under grants to Dick McCombie and Marja Timmermans, faculty/student pairs from universities serving predominantly underrepresented minorities will be awarded summer Fellowships at CSHL. During this intensive residence, Fellows will participate in plant research at the Woodbury Genome Sequencing Center and learn effective teaching strategies at the DNALC. Upon returning to their institutions, Fellows will work with DNALC staff to offer a train-

ing workshop on plant genomics for local biology faculty.

Under a grant to Lincoln Stein, DNALC staff will work with faculty Fellows to develop a *Student Genome Viewer*, an educational interface for annotating and comparing grain genomes. Then, a series of nationwide workshops will be held to train high school and college faculty to use *Genome Viewer* to detect potential errors and inaccuracies in gene predictions. Upon returning to their schools, faculty members will download a set of *ab initio* gene predictions from the DNALC server. They will then guide students in using *Genome Viewer* to evaluate predicted genes and to flag potential errors in exon/intron boundaries and other gene features. Student observations will be sent to database curators, whose suggested fixes and other comments will be shared with them.

DNA Interactive Completed

During the year, we completed *DNA Interactive* (*DNAi*, www.dnai.org), an Internet site to commemorate the 50th anniversary of the discovery of the structure of DNA. As executive producer of this $1.8 million project, the DNALC coordinated contributions from producers and designers in the United Kingdom and Australia—Windfall Films, The Red Green & Blue Company Ltd., The Mill, and The Walter and Eliza Hall Institute. The Internet site was part of a larger DNA project developed by an international collaboration of scientists, educators, and filmmakers. Other DNA products include a five-part TV series airing on PBS in 2004; *DNA: The Secret of Life* book coauthored by Nobel Laureate James D. Watson; a half-hour video for museums and science centers; and DVDs of the television series and *DNAi* teacher resources.

Funded by the Howard Hughes Medical Institute (HHMI), *DNAi* comprises six major topic areas: *Timeline*, *Code*, *Manipulation*, *Genome*, *Applications*, and *Chronicle*. The topics were serially released over a five-month period. By year's end *DNAi* had received 377,000 visits—making it our fastest-growing release to date. The topic areas (with the exception of *Timeline*) loosely follow the *DNA: The Secret of Life* television series. *DNAi* includes five hours of video footage drawn from extensive interviews with more than 70 scientists (including 11 Nobel laureates). More than 150 animations illuminate key experiments in the history of DNA and bring to life the molecular processes that govern DNA replication and expression.

In producing *DNAi*, the Biomedia Group joined the upper echelon of multimedia design groups in the world. The site was produced entirely in Flash MX, which has the advantage of playing its own formatted video files, allowing for seamless integration of a video player into the Internet page itself. When coupled with video encoders, Flash formatting allows us to achieve 100-fold compression of video file size with surprisingly little loss of quality. Flash MX files require only a single plug-in for both animation and video playback, and 98% of Internet users already have a Flash-enabled browser. *DNAi*'s high level of multimedia design and technology integration was recognized when it was named "Site of the Day" by Macromedia, the manufacturer of Flash MX. The site also won *ScientificAmerican.com*'s 2003 Sci/Tech Web Award for biology resources, the Exploratorium's "Ten Cool Sites" Award for education excellence, and the Yahoo! Pick of the Day. We believe that the project now

sets the standard against which other Internet science education resources must be measured.

In November, we released *myDNAi* (www.dnai.org/members), a package of classroom resources and tools that allow teachers to create their own materials. Working closely with expert Teacher Fellows, we developed 15 lesson plans that include objectives, correlations to National Science Education Standards, student worksheets, preparation notes, and supplemental materials. The customized *DNAi* experience begins with the creation of a *myDNAi* homepage. This easily editable page includes a greeting, access to *DNAi* lesson plans, and Internet links. The customized homepage is stored on the DNALC server and is accessed by a unique URL.

myDNAi is also the access point for *Lesson Builder*, our unique editor that allows teachers to build custom lessons from more than 1000 multimedia objects (video, animations, photos, transcripts, text) used to construct the *DNAi* Internet site. A keyword search looks for matches among more than 20 metadata fields that describe each object. Then, using a simple "drag-and-drop" editor, the teacher selects and organizes the multimedia objects into a lesson. Each teacher lesson is then saved on the DNALC server and accessed by students using a unique URL associated with the teacher's profile. A lesson can be modified and saved in different versions for use in different classes. In the final two months of 2003, more than 2400 people had registered as *myDNAi* members, and nearly 400 custom lessons had been developed.

Eugenics Image Archive Nearly Completed

The *Image Archive on the American Eugenics Movement* Internet site was conceived in 1995, soon after the DNALC launched its first primitive home page. We saw the Internet as the perfect medium to introduce students, teachers, and the public to this hidden period in scientific history. After several tries, we were ultimately awarded a two-year grant from the Ethical, Legal, and Social Issues (ELSI) Program of the National Human Genome Research Institute in early 1998; a continuation grant extended support to March 2004.

The *Archive* now contains more than 2200 images of photographs, lantern slides, correspondence, journals, texts, manuscripts, charts, and data. About 900 text-rich images have been transcribed as text-only files, which allow the content to be searched by our database engine. The images represent collections from 11 institutions: American Philosophical Society Library, Philadelphia; Cold Spring Harbor Laboratory Archives, New York; Ellis Island/Statue of Liberty National Monument, New York; International Center of Photography, New York; Max Planck Society Historical Archives, Berlin; Rockefeller University Archive Center, New York; State University of New York at Albany; Truman State University Archives, Missouri; University College, London; University of Tennessee at Knoxville; and University of Virginia.

The content of the *Archive* was significantly bolstered in 2003 by the addition of the Flash MX module *Chronicle* (www.dnai.org/e/index.html), developed as part of the *DNAi* project. Using mental illness as a common thread of eugenic concern during the first half of the 20th century, *Chronicle* fuses a linear narrative with browsable "exhibits" drawn from the *Archive*. The presentation highlights the experiences of five individuals—four who became objects of the eugenic movement's zeal to cleanse society of "bad" genes, and one modern-day heroine who provides a personal account of mental illness and the lesson it holds for living in the "gene age." Paul Lombardo, of the University of Virginia, provides a narrated tour of the people and places involved in the pivotal trial of Carrie Buck, which legalized eugenic sterilization in the United States. In another moving sequence, Benno Müller-Hill takes James Watson on a tour of a gas chamber in Bernberg psychiatric hospital, where the Nazis enforced a "final solution" to mental illness.

Since its public launch in 2000, the *Eugenics Archive* has received 580,000 visitors. Visitation increased 32% in 2003, with nearly a quarter of a million people spending an average of 13 minutes at the site (equivalent to over six person-years of viewing!). The *Archive* has been recognized as a *USA Today* "Hot Site" and one of the Exploratorium's "Ten Cool Sites"; it recently received a high rating in

an in-depth review of Internet sites on the Progressive Era by the Public History Resource Center (publichistory.org/reviews/view_issue.asp?IssueNumber=9). We regularly receive requests for use of *Archive* images from traditional and online publications and broadcast media. Most notably, *Archive* images have been seen in "Race and Membership in American History: The Eugenics Movement" from *Facing History and Ourselves*, "How to Build a Human" broadcast on BBC television, "American Gothic" by Christopher Reardon for *Teaching Tolerance* Magazine, "Race Cleansing in America" by Peter Quinn for *American Heritage* Magazine, "Never Again" by Ward Harkavy for *The Village Voice*, "Breeding Better Citizens" by Valerie Parker for ABC News, and "Race: The Power of an Illusion" by California Newsreel for PBS.

The last in a series of three meetings funded under our National Institutes of Health (NIH) grant, *American Eugenics and the New Biology: Perspectives and Parallels*, was held at Banbury Center October 14–16. *Archive* advisors Garland Allen (Washington University of St. Louis), Elof Carlson (Stony Brook University, New York), Paul Lombardo (University of Virginia), and Steve Selden (University of Maryland) were joined by Nicholas Gillham (Duke University), Conrad Gilliam (Columbia University), Nancy Segal (California State University, Fullerton), and Kay R. Jamison (Johns Hopkins School of Medicine) in speaking on a range of topics designed to provoke thinking about the "old" and potentially "new" eugenics. Talks moved from an in-depth look at the founder of British eugenics, Francis Galton, to eugenic sterilization in the United States, to twin studies and the modern search for genes behind mental illness. The series drew 80 opinion leaders from diverse fields, including family genetics, education, ethics, journalism, government, industry, and philanthropy.

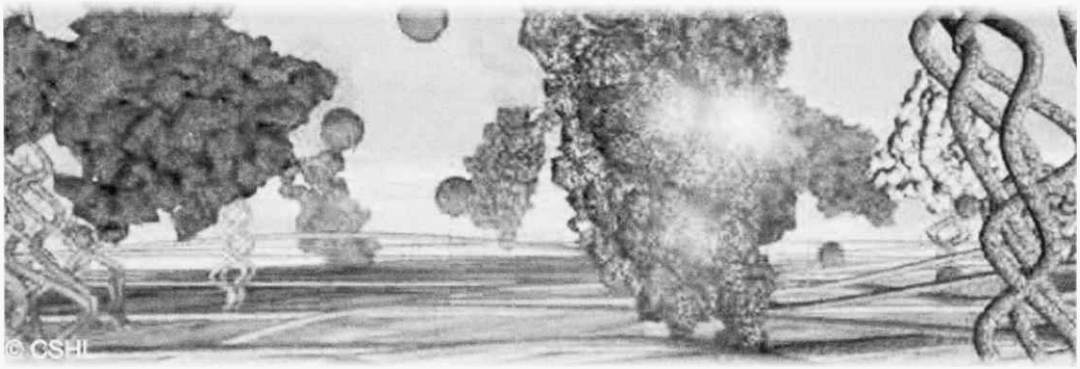
Participants in the *Eugenics and the New Biology: Perspectives and Parallels* Banbury meeting have an informal discussion between sessions.

Inside Cancer in Full Production

Upon completion of the *DNAi* project, the focus of the Biomedia Group turned to *Inside Cancer*—a multimedia Internet site for teachers, students, and family members who want authoritative information on how knowledge of the cancer cell is changing cancer diagnosis and treatment. Funded by an NIH Science Education Partnerships Award (SEPA), *Inside Cancer* is benefiting from the Flash MX technology we mastered in building *DNAi*. With Flash MX, we can now build presentations that focus on telling the story with the most appropriate media, or combination of media types.

Inside Cancer will feature interviews with cancer researchers and other experts to help people understand the complex science and issues of cancer. These interviews will appear both as standalone features on the site and as integrated presentations with the interviewees narrating animations that show the inner workings of cancer cells. More than 15 cancer researchers, clinicians, and epidemiologists have been interviewed to date—including pioneers Judah Folkman, Doug Hanahan, Edward Harlow, Arnold Levine, Harold Varmus, and Robert Weinberg.

The content is broken into five modules. *Hallmarks of Cancer* emphasizes cancer as a genetic dis-



One of the first completed components of the *Inside Cancer* Internet site is the Pathways to Cancer 3-D animation. In this clip, the signal is conducted through the cell membrane, into the cytoplasm. The PDGF receptor is the large molecule just to the right of center.

ease and highlights the common features of a cancer cell. *Causes & Prevention* uses epidemiological data to highlight behaviors and environmental factors that increase cancer risk. *Diagnosis & Treatment* shows how new molecular techniques are being used to diagnose and tailor cancer treatment according to specific genetic changes in the patient's tumor. *Cancer in the Laboratory* introduces major cancer researchers and the importance of their discoveries in understanding cancer at the molecular level. *Pathways to Cancer* is a 3-D tour of a cell that focuses on the signaling pathway through which growth commands are transmitted from the cell surface to the nucleus. All of the modules will be linked through a "molecule menu" that acts as an illustrated glossary for quick reference information on specific genes and proteins involved in oncogenesis.

A major development effort has focused on finishing high-resolution 3-D animations of a cell's signaling pathway for the *Pathways to Cancer* module. These animations, produced with Interactive Knowledge, Inc., enable users to follow a pathway initiated by platelet-derived growth factor (PDGF) to illustrate the key points at which cellular growth control can be lost during oncogenesis. The importance of protein products of proto-oncogenes c-*sis*, *ras*, c-*fos*, c-*jun*, and the role of phosphorylation in the regulation of protein function are illustrated. Since the PDGF receptor is a target for the new Novartis drug Gleevec™, the pharmacological action of cell signaling inhibitors will also be stressed. The signaling animations will be completed early in 2004, when voiceover artist Doug Thomas records a narration track. Doug is the son of Peter Thomas, who did voiceover for the award-winning video, *The Biological Revolution*, produced as part of the Laboratory's centennial celebration. A 12-minute version of the animation, entitled *Cell Signals*, will be produced in wide-screen format for presentation in the DNALC's auditorium.

DNA Science 2nd Edition

In mid-January, we received the first copies of the second edition of our popular lab text, *DNA Science*. The 2nd edition preserves the successful formula of the 1st edition: one part well-tested laboratories and one part insightful, explanatory text. First published in mimeographed form in 1988 and formally published in 1990, this book was largely responsible for bringing DNA experiments within reach of advanced high school and beginning college students.

The core laboratory sequence, developed by myself and Greg Freyer in the laboratory of CSHL Nobel Laureate Richard Roberts, introduces the basic techniques of DNA restriction, transformation, isolation, and analysis—and then applies these techniques to the construction and analysis of a simple recombinant DNA molecule. We resisted the temptation to tinker very much with the laboratories,

since they are the best-tested and most widely used teaching labs available on the basic techniques of gene manipulation. Two new labs were added that focus on gene products: a colorimetric assay for the activity of β-lactamase, the enzyme produced by the ampicillin resistance gene, and expression and purification of green fluorescent protein.

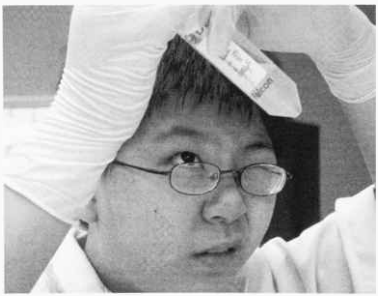
The text portion has been entirely reorganized and updated, increasing from 200 to 300 pages. As before, the narrative takes students behind the scenes of modern research to show them the evolution of concepts and methods. The first three chapters cover essential principles of genetics, and DNA structure and function. The next three chapters introduce small- and large-scale methods for analyzing DNA, culminating in the race to sequence the human genome and new methods for working with hundreds of genes simultaneously. The final two chapters focus on the applications of molecular techniques to understand cancer, human variation, and our emergence as a species. The human genetics chapter also contains the first substantial treatment of the American eugenics movement available in a general biology text.

Although much has changed in biology since the first edition, the ideas and techniques in this book are still the minimum requirements for any degree in DNA manipulation. We hope that *DNA Science* continues to provide a simple roadmap for beginning an exploration of the molecule of life—one that will take on added importance as more and more biology teachers around the world realize the value of giving students freedom to get their hands dirty with DNA.

DNALCs to the North, South, East, and West

Over the years, the DNALC has been the direct model for a number of teaching laboratories established at museums and higher education institutions in the United States and abroad, including Biogen Community Laboratory (Boston, Massachusetts), Tech Museum of Innovation (San Jose, California), Life Science Centre (Newcastle, England), Visible Laboratory (Berlin, Germany), DNA School (Montpelier, France), and Life Learning Center (Bologna, Italy). In 2001, the DNALC initiated a formal program to assist institutions and governments that wish to quickly and efficiently implement a "hands-on" science center devoted to modern biology education. Under the licensing scheme, institutions have transparent access to teaching methods, Internet technology, and intellectual property ("know-how") developed at a cost of more than $25 million.

In 2003, Clemson University and Roberson Museum and Science Center joined the ranks of current licensees: North Shore–Long Island Jewish (LIJ) Health System, Singapore Ministry of Education,



Students in the DNA Learning Lab at the Singapore Science Centre participate in hands-on activities.

and Science Epicenter and DNA Learning Center. Clemson initiated the South Carolina DNA Learning Center with an initial grant of $500,000, with the intent to focus on agricultural genetics. Working with a consortium of local business people and educators at the University of Binghamton, Roberson had raised nearly $1 million to support development of its Life Science Learning Center.

The DNALC *West* facility, adjacent to North Shore–LIJ's clinical testing laboratory in Lake Success, served 2050 students during its first full year of operation. Located 18 miles west of Cold Spring Harbor, this facility was designed to serve students in western Nassau County and New York City. Within a year of signing a licensing agreement, two facilities based on the DNALC model came online in the Republic of Singapore—one to serve teachers and the other to serve students. The DNA Centre at the National Institute of Education (NIE)—with two teaching labs, a bioinformatics lab, and small exhibit space—provided workshops for 625 teachers in 2003. The DNA Learning Lab at the Singapore Science Centre—with dual "Watson" and "Crick" teaching labs surrounded by a "DNA Trail" exhibit—conducted experiments with 6356 visitors and held orientations for 1200 educators. Science Epicenter and DNA Learning Center—across Long Island Sound in Groton, Connecticut—equipped its first teaching lab with the help of Pfizer, Inc. and began offering field trips in the fall.

Real and Virtual Programs Continue to Grow

Thanks to the availability of new lab space at the *West* facility, the number of students participating in lab field trips rose 8% in 2003, to 15,604. The 798 students who participated in summer "DNA camps" represented a similar increase over the previous year. *Gratis* labs and summer camps were provided to nearly 800 students from public schools in Nassau County, Suffolk County, Brooklyn, Manhattan, and the Bronx. We had another busy summer, instructing more than 600 student participants in 26 workshops sited at the DNALC and at Central Islip High School (Suffolk County), Bard High School Early College (Manhattan), and Science Epicenter and DNA Learning Center (Groton, Connecticut). Nearly 250 teachers participated in training workshops (two or more days long) conducted by DNALC staff here, in Singapore, and at locations around the United States. This brings the total number of teachers that we have trained since 1985 to 3500. Nearly 8000 visitors viewed Cablevision's *Long Island Discovery* and *The Genes We Share* exhibit. All told, DNALC visitation reached a new peak of 33,351.

Although DNALC visitation is limited by space, time, and staff, our capacity for "virtual" visitors via the Internet is essentially unlimited. Thus, the family of Internet sites reached through the DNALC's portal, *Gene Almanac*, provide us with a large and growing clientele of students, teachers, medical consumers, and concerned lay people. Traffic at DNALC Internet sites increased 23%, with 4.85 million visitors each spending an average of 11 minutes.

Genetics as a Model for Whole Learning (*GMWL*), our program of in-school instruction and lab field trips for 4th–8th graders, reached 16,000 students in 60 school districts and private schools during the year. Middle school students continued to enjoy a variety of laboratory experiences—from making cell models of gelatin and dry foodstuffs to producing glowing bacteria using GFP (green fluorescent protein) transformation. The instructional staff used the *DNAi Lesson Builder* to create a new computer lab in which students compare fossils of ancient hominids and explore the diversity of mitochondrial DNA. This complements a student's visit to the adjacent exhibit on *Common Origins*.

High school students enjoyed the debut of two new labs. In *Doggy DNA*, students isolate DNA from cheek cells of their favorite pooch (or prepared cells provided by staff) and then use PCR to amplify a short region of the mitochondrial chromosome. Several days after the visit, DNALC staff post the sequences on our *Sequence Server*, where teachers and students can retrieve their canine DNA and use it to study the evolution of domesticated dogs. In the *Genetically Modified Organisms* lab, students use PCR and gel electrophoresis to detect transgenes in genetically modified soybeans and common foodstuffs.

The long-running *Curriculum Study* Program attracted 37 member districts and schools, from which come a majority of student lab participants. *Curriculum Study* members have preferred access to the

DNALC and select from a "menu" of benefits and services—including free and reduced-rate lab field trips, first option on summer workshops, seminars by world class scientists, and teacher in-service training. *Great Moments in DNA Science*, the *Curriculum Study* honors seminar series, hosted 442 students over three evenings of talks presented by CSHL scientists Thomas Volpe, Richard McCombie, and Yuri Lazebnik.

We continued to welcome families from the local area to the DNALC for the *Saturday DNA!* program. In 2003, more than 300 children, teens, and adults participated in 90-minute laboratory- or computer-based sessions offered by DNALC staff. During lab sessions, participants compared DNA samples at mock crime scenes, mapped DNA mutations, and experimented with jumping genes. In computer-based sessions, participants learned how to create scientific animations, explored molecular biology resources on the Web, and searched for meaning in DNA sequences. In addition, more than 900 members of the public attended "DNA at 50," a series of nine special lectures given by CSHL Chancellor James Watson to commemorate the 50th anniversary of his own discovery of the structure of DNA.

Pfizer Leadership Institute

The highlight of the summer was the Pfizer *Leadership Institute in Human and Plant Genomics*. Nicknamed "DNA Boot Camp," the three-week institute drew together 19 of the very best biology teachers from around the United States, as well as five teachers from the Republic of Singapore. Staying on the CSHL campus is a dream come true for these teachers. Here, they literally walk in the shadows of heroes—living, eating, and breathing science. For most, it is a defining moment in their careers. The participating teachers have already implemented major genetics/biotechnology teaching programs at their schools prior to attending the Pfizer *Institute*. The *Institute* honors their past creativity and prepares them to take their innovative classroom teaching and peer training to even higher levels.

The 2003 *Institute* focused on the study of human and plant genomes, and included a section on site-directed mutagenesis and genetically altered food crops. All topics were addressed in a three-pronged approach of lectures/seminars, wet-labs, and computer work. Five CSHL research scientists gave seminars on topics that extended participants' understanding of the course materials. Participants also visited CSHL's Uplands Farm field station for plant genetics and the newly completed Genome Sequencing Center. Finally, teachers were provided with independent time to begin developing curriculum materials for use in their own classrooms, as well as for the training of colleagues in their local regions.

A survey of past *Leadership* participants revealed that they, indeed, are living up to their role as educational leaders. Participants reported that they had provided 227 hours of in-service training for 400 precollege faculty—an average of 11 hours and 19 faculty trainees per participant. These events provided faculty hands-on experience in many lab and computer methods, including gel electrophoresis (211 teachers), bioinformatics (111 teachers), PCR (98 teachers), protein purification (50 teachers), and bacterial transformation (34 teachers). *Leadership* faculty also reported receiving more than $80,000 in funding to purchase lab equipment and supplies, to provide in-service training, and to attend professional meetings.

2003 Leadership Institute participants and DNALC staff.

Staff and Interns

In 2003, we welcomed two new members to our Administrative staff: Carolyn Reid and Mary Lamont. Carolyn joined us in May as an Administrative Assistant at the DNALC, helping with schools administration and our *Saturday DNA!* program. A Long Island native, Carolyn has experience in the travel, cable television, and publishing industries. Mary began in September as an Administrative Assistant for the growing operation at the DNALC *West*. Prior to joining CSHL, Mary worked in entertainment management, magazine publishing, and insurance-related companies.

Mary Lamont and Carolyn Reid joined the administrative staff in 2003.

In 2003, we bid farewell to three staff members. After making valuable contributions to the *DNA Interactive* project, science writer Beverly Tomov left in September to look after her new baby, Sophia. Designer Karwai Pun also left the *Biomedia* Group, but continues her relationship with CSHL as a member of the Media Arts & Visualization Department. After returning to the DNALC following maternity leave, Tricia Maskiell took a position as a 6th grade science specialist at Oldfield Middle School, in the neighboring Harborfields School District.

High school interns continue to provide key support for our teaching labs, as well as conducting independent research projects under the direction of Scott Bronson and Jennie Aizenman. Intern Michelle Louie (Kings Park High School) was selected as an Intel semifinalist for her work on tagging *Arabidopsis* genes of unknown function with green fluorescent protein (GFP). Benjamin Blond (Syosset High School) is attempting to use RNA interference (RNAi) to "silence" a *C. elegans* gene believed to be involved in the aging process. Two DNALC *West* interns moved on to pursue higher-level research: Robert Weintraub (Walt Whitman High School) is working on DNA polymorphisms in *Arabidopsis* with Zachary Lippman at CSHL. Alinea Noronha (Herricks High School) was accepted into the Young Investigators Internship at the Research Institute of NS–LIJ Health System.

Regina Hui (Northport High School), Ariel Gitlin (Cold Spring Harbor High School), and Watson School student Elizabeth Thomas continued to assist the Biomedia group with Internet site development.

Joining the intern program in 2003 were Carisa Bautista (Cold Spring Harbor High School), Joe Hakoopian (Walt Whitman High School), Zach Goldberg (Half Hollow Hills East High School), Amy Richards (Kings Park High School), George Roche (Cold Spring Harbor High School) and Greg Rosen (MacArthur High School). Jason Wittenstein (Herricks High School) and Zeba Izhar (Queensborough Community College) joined the intern crew at DNALC *West*. Several interns returned from college to assist with summer workshops: Yan Liang Huang (Notre Dame University), Janice Lee (Boston University), and Alex Witkowski (SUNY Albany).

In August, we bid farewell to a number of interns as they began their freshman year at college: Lara Abramowitz (Half Hollow Hills West High School) at the University of Rochester; Kunal Kadakia (Syosset High School) at Northwestern University; Shirish Kondabolu (Half Hollow Hills West High School) at the University of Rochester; Jared Winoker (Syosset High School) at Cornell University, and Jonathan Mogen (Half Hollow Hills West High School) at Brown University. Pushpa Abraham (Kings Park High School) traveled to India to attend medical school, and Rebecca Yee entered a Masters-level program in genetic counseling at Virginia Commonwealth University.

Dave Micklos
Executive Director

2003 Workshops, Meetings, and Collaborations

January 2	Site visit by Barry Aprison, Museum of Science and Industry, Chicago, Illinois
January 13	Site visit by Barbara Suhr, New York Public Library, New York, New York
January 17	Site visit by Phil Lamesch, Harvard Medical School, Boston, Massachusetts
January 21	Site visit by Anna Nasmyth and Gustav Ammerer, Mendel Museum of Genetics, Brno, Czech Republic
January 24–25	European Molecular Biology Laboratory Education Advisory Board Meeting, Heidelberg, Germany
January 24	Site visit by Mark Bloom, Biological Sciences Curriculum Study, Colorado Springs, Colorado
January 25	*DNA at 50: Finding the Double Helix*, talks by James D. Watson, DNALC
January 27	Site visit by the Bruce Museum, Greenwich, Connecticut
January 29	National Institute of Social Sciences Issues Discussion Group, Colony Club, New York, New York
January 29–31	National Coalition for Health Professional Education in Genetics Annual Meeting, Bethesda, Maryland
January 31	Site visit to Roberson Museum and Science Center, Binghamton, New York
February 4	Site visit to Molloy College, Rockville Centre, New York
February 8	*DNA at 50: Finding the Double Helix*, talks by James D. Watson, DNALC
February 14	Site visit by Mark Hertle, Precollege Science Education Program, Howard Hughes Medical Institute, Chevy Chase, Maryland
February 15	*DNA at 50: Finding the Double Helix*, talks by James D. Watson, DNALC
February 20–21	National Institutes of Health Science Education Partnerships Award Directors Meeting, La Jolla, California
February 25–27	National Institutes of Health Science Education Partnerships Award Review Panel, Bethesda, Maryland
February 27–March 1	*Meeting the Challenges in Emerging Areas: Education Across the Life, Mathematical, and Computer Sciences* Meeting, Bethesda, Maryland
February 28	Site visit by Jon King, National Human Genome Research Institute, Bethesda, Maryland
March 5	Site visit by Hutton House faculty and members, C.W. Post Campus, Long Island University, Brookville, New York
March 8	*Saturday DNA!* Seminar, DNALC
March 17–24	Teacher-training workshops, National Institute of Education and Singapore Science Centre, Singapore
March 19–22	*Museums and the Web* Meeting, Charlotte, North Carolina
March 22	*Saturday DNA!* Seminar, DNALC
March 25	*Great Moments in DNA Science* Honors Students Seminar, CSHL
March 27–30	Exhibit at National Science Teachers Association Annual Meeting, Philadelphia, Pennsylvania
March 28	Site visit by News 12 Long Island, to film high school field trip
March 31	Site visit by Kathy Belton, Audrey Cohan, Valerie Collins, Jodi Kilgannon, Ed Thompson, and Ron Zanni, Molloy College, Rockville Centre, New York
April 1	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 2	Eugenics Seminar at Lycoming College Genome Symposium, Williamsport, Pennsylvania
April 3	Site visit by Jennifer Cordi and Judy Cohen, Bard High School Early College, Manhattan, New York
	Presentation at Sayville High School Career Café, West Sayville, New York
April 5	*Saturday DNA!* Seminar, DNALC
April 8	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 11	Site visit by Fran Balkwill, Barts & The London, Queen Mary's School of Medicine and Dentistry, London, United Kingdom
April 15	Site visit by Callie Brunelli, Aubrey Clark, Denise Gay-Alden, Kristen Gordon, Jim Griffin, Jim Lee, and Nancy Scales, Roberson Museum and Science Center, and Tom O'Brien and Theresa Partell, SUNY Binghamton
April 16	Science Epicenter and DNA Learning Center Board Meeting, New London, Connecticut
April 19	*Saturday DNA!* Seminar, DNALC
April 30	National Institute of Social Sciences Issues Discussion Group, Harvard Club, New York, New York
May 3	*Saturday DNA!* Seminar, DNALC
May 6	Presentation to Academy of Professional Law Enforcement, CSHL

May 13	Site visit by members of the Huntington Rotary Club, Huntington, New York
May 17	*Saturday DNA!* Seminar, DNALC
May 19	Site visit by Robert Ballard, Richard Hilderman, and Calvin Schoulties, Clemson University, Clemson, South Carolina
May 22	Sayville School Business Advisory Board Meeting, Sayville High School, West Sayville, New York
May 27	Site visit by Estelle Perera, The Tech Museum of Innovation, San Jose, California
	National Institute of Social Sciences Annual Board Meeting, Colony Club, New York, New York
May 30	Site visit by participants in the CSHL Symposium *The Genome of Homo Sapiens*
May 31	Saturday DNA! Seminar, DNALC
June 3	Site visit by Linnea Fletcher, Austin Community College, and Sue Gravett, Brandon Janes, and Charles Lutz, Austin Chamber of Commerce, Texas
June 9	*DNA: The Secret of Life* book signing, James D. Watson, DNALC
June 10	Site visit by Jean Caron and Carol Milne, Science Epicenter and DNA Learning Center, New London, Connecticut
June 13	Site visit by Jim Lee, Ann Van Atta, Miranda Green-Barteet, and Nancy Scales, Roberson Museum and Science Center, Binghamton, New York
June 14	*Saturday DNA!* Seminar, DNALC
June 16–20	National Science Foundation Advisory Panel Workshop, DNALC
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, CIIT Center for Health Research, Research Triangle Park, North Carolina
June 23–27	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Blood Center of Southeastern Wisconsin, Milwaukee
June 26	Site visit by Horst Saalbach and Berndt Kynast, Faustus Forschungs Compagnie, Leipzig
June 26–July 2	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
July 1–3	DNA polymorphisms workshop, International Genetics Congress, Melbourne, Australia
July 6–11	International Congress of Genetics, Melbourne, Australia
July 7–11	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Green Genes Workshop, DNA Learning Center *West*
	DNA Science Minority Workshop, Central Islip High School
July 7–25	Pfizer *Leadership Institute in Human and Molecular Genomics*, DNALC
	Training for Singapore collaborators, Muhammad Shahrin, Nai Sok Khoon Karine, Elaine Tan Pei Lee, and Florence Francis
July 8	Seminar at Human Genetics and Global Healthcare Symposium, Melbourne, Australia
July 14–18	*Fun With DNA* Workshop, DNA Learning Center *West*
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNALC
	Training for Science Epicenter and DNA Learning Center collaborators, Pat Quinn and Nancy Scales
July 15	Site visit by Alan Fleischman and Leslie Goldman, New York Academy of Medicine, New York, and Lawrence Sherr, North Shore–Long Island Jewish Health System, Manhasset, New York
July 18	Site visit by the Alliance for Graduate Education and Professionals, Stony Brook University, New York
July 21	Presentation at National Institutes of Health workshop, *Genes, Schemes, and Molecular Machines*, Schenectady, New York
July 21–25	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNA Learning Center *West*
	DNA Science Workshop, DNALC
	DNA Science Minority Workshop, Bard High School Early College
	DNA Science Workshop, Science Epicenter and DNA Learning Center, New London, Connecticut
July 28–August 1	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	DNA Science Workshop, DNA Learning Center *West*
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Coriell Institute for Medical Research, Camden, New Jersey

August 4–8	*Fun With DNA* Workshop, DNALC
	Fun With DNA Workshop, DNA Learning Center *West*
	World of Enzymes Workshop, DNALC
	Genomic Biology and PCR Workshop, DNALC
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Harbor-UCLA Research and Education Institute, Torrance, California
August 4–22	Training for Singapore collaborators, Chow Wai Hoong, Ramiah Kamala, and Yam Wei Ling Agnes
August 11–15	*Fun With DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNALC
	Genomic Biology and PCR Workshop, DNA Learning Center *West*
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Kaiser Permanente Center for Health Research, Portland, Oregon
	Training for Science Epicenter and DNA Learning Center collaborator, Nancy Scales
August 12	Site visit by alumni group from Brandeis University, Waltham, Massachusetts
August 13	Site visit by Karl Kuchler, University of Vienna, Austria
August 18–22	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
	DNA Science Workshop, DNA Learning Center *West*
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Rockefeller University, New York, New York
August 25–29	*Fun With DNA* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	Genomic Biology and PCR Workshop, DNALC
September 3	Site visit by Henry Yang, Beijing Genomics Institute, China
September 9	Dedication of Joan and Arthur M. Spiro Auditorium, *Nature, Nurture & Mental Illness*, talks by Matt Ridley, Kay Redfield Jamison, and Andrew Solomon, DNALC
September 10	Presentation at Melbourne Museum, Australia
September 20	*Saturday DNA!* Seminar, DNALC
September 25	Eugenics seminar at the New York Public Library, New York
September 26	Site visit and curriculum development meeting, Southampton College–Long Island University, New York
September 30	*Inside Cancer* interview, Arnold Levine, Princeton University, Princeton, New Jersey
October 1–3	Site visits to China Academy of Science and Technology and Natural History Museum, Beijing, China
October 2	Sayville School Business Advisory Board Meeting, Sayville High School, West Sayville, New York
October 4	*Saturday DNA!* Seminar, DNALC
October 6	Dedication of James D. Watson Institute of Genome Sciences, Zhejiang University, Hangzhou, China
October 8–11	Exhibit at National Association of Biology Teachers Annual Meeting, Portland, Oregon
October 11	Site visit and tour by students and faculty from the Center for Talented Youth, Johns Hopkins University, Baltimore, Maryland
October 13–16	National Institutes of Health ELSI conference, *American Eugenics and the New Biology: Perspectives and Parallels*, Banbury Center, CSHL
October 15	Site visit to Roberson Museum and Science Center, Binghamton, New York
October 16–17	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, Editorial Working Group Meeting
	Bio 21: Teaching Biology With Bioinformatics Meeting, University of North Carolina at Chapel Hill
October 18	*Saturday DNA!* Seminar, DNALC
October 20–23	Site visit by Yan Yaw Kai, National Institute of Education, Singapore
October 22	Site visit by members of the National Institute of Social Sciences, New York, New York
October 24	*Inside Cancer* interview, Harold Varmus, Memorial Sloan-Kettering Cancer Center, New York
	Site visit by Dixie Scovel and Tony Wills, *Newsday*, Melville, New York
October 28–31	Site visit to International Centre for Life, Newcastle, National Centre for Biotechnology Education, Reading, and The Mill, London, United Kingdom
October 29	Teacher-training workshop, Erasmus Hall High School and STAR High School, Brooklyn, New York

November 1	*Saturday DNA!* Seminar, DNALC
November 3	Site visit by Arthur Spiro, CSHL Trustee and DNALC Committee Chairman, and James and Marcia Barker, Calvin Schultheis, Mendel Bouknight, and Robert Ballard, Clemson University, Clemson, South Carolina
	Inside Cancer interview, Larry Norton, Memorial Sloan-Kettering Cancer Center, New York
November 4	*Inside Cancer* interview, Bob Weinberg, Whitehead Institute, Cambridge, Massachusetts
	Inside Cancer interview, Stanley Korsmeyer, Dana-Farber Cancer Institute, Boston, Massachusetts
	Teacher-training workshop, Erasmus Hall High School and STAR High School, Brooklyn, New York
November 5	Site visit by Don Colbert, State University of New York at Binghamton
	Inside Cancer interview, Ed Harlow, Harvard Medical School, Boston, Massachusetts
	Teacher-training workshop, Biogen, Cambridge, Massachusetts
November 6	*Inside Cancer* interview, Marianne Berwick, Memorial Sloan-Kettering Cancer Center, New York
November 7	Videoconference with James D. Watson and National Institute of Education, Singapore
November 10–22	Teacher-training workshops, National Institute of Education, Singapore
November 11	Site visit by Jiaan Cheng, Zhejiang University, Hangzhou, China, and Sun Zhongsheng, Cornell University, Ithaca, New York
November 15	*Saturday DNA!* Seminar, DNALC
November 19	American Association of University Women Excellence in Science Awards Reception and Meeting, DNALC
	Teacher-training workshop, Erasmus Hall High School and STAR High School, Brooklyn, New York
November 20	Site visit by Curt Engelhorn, Monte Carlo, Monaco
	Site visit by Kent Anderson and Bette Phimister, *New England Journal of Medicine*, Waltham, Massachusetts
December 1–12	Training for Singapore collaborators, Wong Poh San, Ng Huey Bian, Evan Yap Boon Heng, Teo Lay Yen, Tan Wei Ling, Wong Mei Leng, and Tan Woei Yng
December 2	Site visit by Clare Lin, national winner of the GenETHICS Competition, Melbourne, Australia
December 13	*Saturday DNA!* Seminar, DNALC
December 15	Presentation at Garcia Center open house and science fair, Queens College, Flushing, New York
	Inside Cancer interview, Richard Stanley, The Albert Einstein College of Medicine, Bronx, New York
December 17	Site visit by Bruce Kovner, Chairman of the Board of Trustees of The Juilliard School, New York, New York
December 27	*Saturday DNA!* Seminar, DNALC

Sites of Major Faculty Workshops 1985–2003

Key: High School **College** *Middle School*

ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	University of Alaska, Fairbanks	1996
ARIZONA	Tuba City High School	1988
ARKANSAS	Henderson State University, Arkadelphia	1992
CALIFORNIA	**Foothill College, Los Altos Hills**	**1997**
	University of California, Davis	1986
	San Francisco State University	**1991**
	University of California, Northridge	**1993**
	Canada College, Redwood City	1997
	Pierce College, Los Angeles	**1998**
	California Lutheran University, Thousand Oaks	1999
	Laney College, Oakland	1999
	California State University, Fullerton	**2000**
	Salk Institute for Biological Studies, La Jolla	2001
	Contra Costa County Office of Education, Pleasant Hill	2002
	Harbor-UCLA Research & Education Institute, Torrance	2003
COLORADO	Colorado College, Colorado Springs	1994
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
DISTRICT OF COLUMBIA	**Howard University**	**1992, 1996**
FLORIDA	North Miami Beach Senior High School	1991
	University of Western Florida, Pensacola	1991
	Armwood Senior High School, Tampa	1991
	University of Miami School of Medicine	2000
GEORGIA	Fernbank Science Center, Atlanta	1989
	Morehouse College, Atlanta	**1991, 1996**
	Morehouse College, Atlanta	1997
HAWAII	Kamehameha Secondary School, Honolulu	1990
ILLINOIS	Argonne National Laboratory	1986, 1987
	University of Chicago	**1992, 1997**
INDIANA	Butler University, Indianapolis	1987
IDAHO	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
MAINE	Bates College, Lewiston	1995
	Foundation for Blood Research, Scarborough	2002
MARYLAND	Annapolis Senior High School	1989
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	St. John's College, Annapolis	1991
	University of Maryland, School of Medicine, Baltimore	**1999**
	National Center for Biotechnology Information, Bethesda	2002
MASSACHUSETTS	Beverly High School	1986
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	Winsor School, Boston	1987
	Boston University	**1994, 1996**
	Whitehead Institute for Biomedical Research, Cambridge	2002
	Biogen, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990, 1991
MISSOURI	Washington University, St. Louis	1989
	Washington University, St. Louis	**1997**
	Stowers Institute for Medical Research, Kansas City	2002
NEW HAMPSHIRE	St. Paul's School, Concord	1986, 1987
	New Hampshire Community Technical College, Portsmouth	**1999**
NEVADA	University of Nevada, Reno	1992
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
NEW YORK	Albany High School	1987
	Bronx High School of Science	1987

	Columbia University, New York	1993
	Cold Spring Harbor High School	1985, 1987
	DeWitt Middle School, Ithaca	1991, 1993
	DNA Learning Center	1988–1995, 2001–2003
	DNA Learning Center	**1990, 1992, 1995, 2000**
	DNA Learning Center	1990–1992
	Fostertown School, Newburgh	1991
	Huntington High School	1986
	Irvington High School	1986
	Junior High School 263, Brooklyn	1991
	Lindenhurst Junior High School	1991
	Mt. Sinai School of Medicine, New York	1997
	Orchard Park Junior High School	1991
	Plainview–Old Bethpage Middle School	1991
	State University of New York, Purchase	1989
	Stony Brook University, New York	1987–1990
	Titusville Middle School, Poughkeepsie	1991,1993
	Wheatley School, Old Westbury	1985
	US Military Academy, West Point	**1996**
	Stuyvesant High School, New York	1998–1999
	Trudeau Institute, Lake Saranac	2001
	Rockefeller University, New York	2003
NORTH CAROLINA	North Carolina School of Science, Durham	1987
	CIIT Center for Health Research, Triangle Park	2003
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	North Westerville High School	1990
OKLAHOMA	School of Science and Mathematics, Oklahoma City	1994
	Oklahoma City Community College	**2000**
	Oklahoma Medical Research Foundation, Oklahoma City	2001
OREGON	Kaiser Permanente-Center for Health Research, Portland	2003
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
SOUTH CAROLINA	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	1988
TEXAS	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Taft High School, San Antonio	1991
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999**
	Austin Community College-Rio Grande Campus	2000
	Southwest Foundation for Biomedical Research, San Antonio	2002
UTAH	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998**
	University of Utah, Salt Lake City	**2000**
VERMONT	University of Vermont, Burlington	1989
VIRGINIA	Eastern Mennonite University, Harrisonburg	1996
	Jefferson School of Science, Alexandria	1987
	Mathematics and Science Center, Richmond	1990
	Mills Godwin Specialty Center, Richmond	1998
WASHINGTON	**University of Washington, Seattle**	**1993, 1998**
	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Marquette University, Milwaukee	1986, 1987
	University of Wisconsin, Madison	1988, 1989
	Madison Area Technical College	**1999**
	Blood Center of Southeastern Wisconsin, Milwaukee	2003
WYOMING	University of Wyoming, Laramie	1991

AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
CANADA	Red River Community College, Winnipeg, Manitoba	1989
ITALY	Porto Conte Research and Training Laboratories, Alghero	1993
	International Institute of Genetics and Biophysics, Naples	1996
PANAMA	**University of Panama, Panama City**	**1994**
PUERTO RICO	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SINGAPORE	National Institute of Education	2001–2003
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995


COLD SPRING HARBOR
LABORATORY PRESS

2003 PUBLICATIONS

SERIALS

Genes and Development, Volume 17, 1–3170

Genome Research, Volume 13, 1–2786

Learning and Memory, Volume 10, 1–540

Protein Science, 12, 1–2886

RNA, Volume 9, 1–1592

Cold Spring Harbor Symposia on Quantitative Biology: Volume 67: *The Cardiovascular System*

LABORATORY MANUALS AND HANDBOOKS

RNAi: A Guide to Gene Silencing, Greg Hannon (ed.)

PCR Primer (2nd ed.), Carl Dieffenbach and Gabriela Dveksler

Purifying Proteins for Proteomics: A Laboratory Manual, Richard Simpson (ed.)

Lab Math: A Handbook of Measurements, Calculations, and Other Quantitative Skills for Use at the Bench, Dany Spencer Adams

TEXTBOOKS

DNA Science: A First Course (2nd ed.), David Micklos and Greg Freyer

Molecular Biology of the Gene (5th ed.), James D. Watson, Richard Losick, Michael Levine, Alex Gann, Tania A. Baker, and Stephen P. Bell

MONOGRAPHS

Prion Biology and Diseases (2nd ed.), Stanley B. Prusiner (ed.)

GENERAL INTEREST AND CHILDREN'S BOOKS

The Molecular Gaze: Art in the Genetic Age, Dorothy Nelkin and Suzanne Anker

Inspiring Science: Jim Watson and the Age of DNA, John Inglis, Joseph Sambrook, and Jan Witkowski (eds.)

George Beadle, An Uncommon Farmer: The Emergence of Genetics in the 20th Century, Paul Berg and Maxine Singer

Reader's Guide: Abraham Lincoln's DNA and Other Adventures in Genetics, Philip R. Reilly

OTHER

CSHL Annual Report 2002: Yearbook Edition

CSHL Annual Report 2002

Banbury Center Annual Report 2002

Watson School of Biological Sciences Annual Report 2002

WEB SITES

Dna-microarrays.org, a companion Web site to *DNA Microarrays: A Laboratory Manual*

Proteinsandproteomics.org, a companion Web site to *Proteins and Proteomics: A Laboratory Manual*

MEDIA

"Selected Songs for Cynical Scientists" (CD), Ron Laskey

The journal publishing program

A selection of recently published books

COLD SPRING HARBOR LABORATORY PRESS

Cold Spring Harbor Laboratory Press contributes in important ways to the Laboratory's educational mission, with an international publishing program for three distinct audiences. Research journals, monographs, handbooks, and laboratory manuals assist the continuing professional education of working scientists and help train graduate students for advanced degrees. Textbooks are created for college undergraduates majoring in the life sciences. In addition, general interest and historical books are published for readers not technically trained in science but interested in its practice and personalities and how research advances are influencing medicine, industrial development, and social policy.

Financially, the year was a disappointing one for the Press. Total income from the publishing program increased by 11%, but there was a net operating deficit after deductions for depreciation and Laboratory overhead. The journal program performed to expectation, but several factors combined to lower the margins on book sales, including pressure for increased discounts from retail and wholesale distributors, declines in sales of larger more expensive books, and increased sales in foreign markets where deep discounts are required.

Books and Electronic Media

Scientists worldwide rely on technical manuals published by the Press, with their outstanding reputation for clarity and accuracy. The list of more than 25 current titles includes *Molecular Cloning*, the world's most widely used source of techniques for working with DNA. The three new manuals published in 2003 included Richard Simpson's *Purifying Proteins for Proteomics*, a companion to his previous proteomics manual, and a second edition of the best-selling guide to PCR technology *PCR Primer* by Carl Dieffenbach and Gabriella Dveksler. The most immediate success was *RNAi* (RNA interference), which describes a new, powerful, and swiftly adopted way of silencing genes. Cold Spring Harbor scientists have been pioneers of RNAi, and one of them, Gregory Hannon, edited what became the first authoritative book on the biology of the interference process and its practical applications.

Laboratory procedures such as RNAi require specialized equipment and reagents. Ten years ago, we assembled databases of such materials and their suppliers and created directories that were published in print and on-line. After becoming the foundation of a private company that was bought by the Internet commerce pioneer, SciQuest.com, the BioSupplyNet databases were reaquired by the Press late in 2003. They are being retooled, enhanced, and integrated into our techniques publishing program.

Recognizing the enthusiasm of scientists for information available on-line, we have created in recent years a number of Web sites that support individual books by providing additional content not available in print and downloadable versions of the artwork. We also continue to collaborate with the National Center for Biotechnology Information in presenting books in an on-line format, this year adding the successful advanced textbook *Essentials in Glycobiology* first published in 1999.

Our series of short, inexpensive handbooks continues to be very popular, and Kathy Barker's books—*At The Bench*, for laboratory novices, and *At The Helm*, for aspiring principal investigators—maintained strong sales. The collection grew in 2003 with the addition of Dany Spencer Adams' *Lab Math*, a book with a purpose spelled out in the subtitle: *A Handbook of Measurements, Calculations, and Other Quantitative Skills for Use at the Bench*. Life scientists are not always confident in their mathematical abilities, and it was satisfying to see the book being eagerly seized on our exhibit stands.

The Cold Spring Harbor Monograph Series, begun in 1970 at Jim Watson's instigation with a volume entitled *The Lactose Operon*, has expanded to 41 volumes, many of them classics of molecular biology. The books' appearance had not changed in 30 years and this year, the interior design was modified slightly and a new cover design imposed, which retains the familiar black spine of the original, but now includes a striking color image. The first book to benefit was the second edition of *Prion Biology and Diseases*, the definitive monograph on the causative agents of bovine spongiform encephalopathy and other degenerative disorders, edited by the discoverer of prions, Nobel prize win-

ner Stanley Prusiner. By chance, its publication coincided almost exactly with the first appearance in the United States of "mad cow disease."

Textbooks for undergraduates are a recent addition to the publishing program. Both titles published in 2003 had strong Cold Spring Harbor connections. The second edition of *DNA Science*, by David Micklos and Greg Freyer, combines laboratory protocols developed at the Dolan DNA Learning Center with a lively narrative about current molecular biology and its applications in medicine and industry. A unique educational contribution, it has been extensively adopted in foundation courses in high schools and junior colleges in the United States and abroad.

James Watson's classic textbook *Molecular Biology of the Gene* was first published in 1965 and for 25 years was the book of choice for advanced undergraduate teaching in genetics. The fourth edition was published in 1987, and two years ago, we undertook to publish a completely revised edition in 2003, recruiting as authors Richard Losick from Harvard University, Tania Baker and Stephen Bell from Massachusetts Institute of Technology, Alex Gann from Cold Spring Harbor Laboratory, and Michael Levine from University of California, Berkeley. Our partner in this venture was the book's original publisher, Benjamin Cummings, a textbook-specialist division of Pearson Plc. with national and international marketing strengths. The time specified for this giant task was short, but the authors, with a large support team from Cold Spring Harbor, Benjamin, and other companies, sacrificed family and professional commitments in order to achieve the goal and the book was published to acclaim in December 2003. Advance sales were brisk, and we anticipate that the new and handsome edition will restore the book to a central place in the teaching curriculum.

In a year in which many scientific conferences, cultural events, and news reports around the world were devoted to the 50th anniversary of Watson and Crick's proposal for the structure of DNA, a book was assembled in some secrecy to honor Jim Watson, for whom 2003 was also a 35th year of distinguished Laboratory leadership. With more than 40 essays by people who have worked with him in all kinds of contexts, *Inspiring Science: Jim Watson and the Age of DNA*, edited by myself, Joe Sambrook, and Jan Witkowski, succeeded handily in presenting a multidimensional sketch of Dr. Watson and his diverse and extraordinary achievements. The first copy of the book was presented to Jim at a dinner held during a meeting at Cold Spring Harbor to which many of the Laboratory's scientist-alumni returned.

Another notable, if more conventionally structured, biographical portrait was created by Paul Berg and Maxine Singer in *An Uncommon Farmer*, the life of George Beadle, the Nobel prize winning corn geneticist who created a powerhouse department at Caltech and became President of the University of Chicago. Their book was widely reviewed and it was gratifying to see equally warm reviews by scientists close to the subject matter and professional historians of science—two groups who do not always share the same opinion of what they read.

Our general interest books included *The Molecular Gaze: Art in the Genetic Age*, a strikingly illustrated survey of artists' responses to the exhilaration and anxieties provoked by gene technologies. It was written by Suzanne Anker, a professor of art history at New York's School of Visual Arts, and the distinguished sociologist of science, Dorothy Nelkin. Sadly, Dot died in June after a short illness and did not see the published book, but she was highly involved in its creation.

The power of a book about science that truly engages the public was vividly illustrated by Ann Arbor's community reading program featuring our 2000 book *Abraham Lincoln's DNA and Other Adventures in Genetics*. For three months, the book and its contents were the focus of discussion groups, university lectures, high school projects, and public appearances by author Philip Reilly, with the result that at times in local stores, it competed successfully in sales with the adventures of Harry Potter.

During the year as a whole, a larger number of Cold Spring Harbor books—79,000 copies—were sold than ever before. This achievement results in part from strengthened relationships with major book wholesalers, high street bookshops, book clubs, and on-line stores such as Amazon.com. Our customer service and warehouse staff rose to the challenge, assisted by improvements in technology such as electronic data interchange that allows automated order processing for corporate customers.

These sales channels widen our audience but exact a price in the form of larger discounts, smaller margins, and, in some cases, unpredictable numbers of returned copies, factors that contributed to missed income projections from U.S. sales. The expansion of international sales that began several

free of charge. As a not-for-profit publisher, we support the principle of open access to an extent that maintains our journals' growth as high-quality publications. New journals have been launched on the business model and so far require support by foundation funds or private wealth.

The development of centralized, free databases of published papers is important in making research results available to both working scientists and the public. Papers published in Cold Spring Harbor journals more than one year previously are already freely accessible at the journals' Web sites, and these papers also appear on the PubMedCentral database at the National Library of Medicine. For *Genome Research*, the availability of these archive papers was reduced to six months and other journals will follow suit if subscriptions are not threatened. Agreements were made with two international initiatives, HINARI and AGORA, to make the Cold Spring Harbor journals freely available on-line to scientists in low- and lower-middle-income countries. In addition, an agent was engaged in China, with the responsibility for providing reduced-price subscriptions to institutions there.

For the Cold Spring Harbor Laboratory Press, in summary, 2003 was a year of financial challenges but significant accomplishments in our mission of bringing important and reliable scientific information to a broad audience of scientists, students, and the public worldwide.

Staff

The staff members of the Press (as of December 2003) are listed elsewhere in this volume. They deserve much credit for the care and commitment they bring to their duties and it is a pleasure to work with them.

In 2003, we welcomed six new colleagues: Colleen Becker, Laureen Connell, Maria Falasca, Keith Kalinoski, Mary Mulligan, Corrisa Salzman, and Rena Steuer. We also said goodbye to Elizabeth Fitzpatrick, Candice Foresta, Keith Kalinoski, Michele McDonough, Maureen Megonigal, and Nora Rice.

At a special luncheon in September, we honored Dorothy Brown as she retired from the Laboratory staff after 22 years of dedicated and cheerful service. During this time, Dotty edited scores of Cold Spring Harbor books and for many years has had the major share of responsibility for preparing the contents of the Laboratory's Annual Reports for publication. As a plaque in St Paul's Cathedral says of its architect: "If you seek a memorial, look around you."

Alexander Gann, who joined the Press as Senior Editor in 1999, assumed the new role of Editorial Director, with responsibility for managing the acquisition of new books. He joined a group of senior staff whose talent and dedication are essential elements in continued growth and innovation at the Press: Jan Argentine, Editorial Development Manager; Ingrid Benirschke, Marketing Manager; Kathy Cirone, Circulation Manager; Kathryn Fitzpatrick, Marketing Manager; Nancy Hodson, Operations Manager; Geraldine Jaitin, Customer Service Manager; Bill Keen, Finance Director; Guy Keyes, Sales Manager; Marcie Siconolfi, Advertising Manager; Linda Sussman, Journal Production Manager; Denise Weiss, Book Production Manager; and the editors of our journals, Terri Grodzicker at *Genes & Development* and Laurie Goodman at *Genome Research*. And it is a pleasure as always to thank my Executive Assistant, Elizabeth Powers, for her continued grace in juggling tasks and responsibilities in our office.

In a year that was unsatisfactory from a financial perspective, much pleasure could be taken from the fact that a large proportion of our new titles were reviewed in prominent science journals, and in very flattering terms. Since there are few sources of science book reviews these days, and their space is limited, this outcome demonstrates the special regard scientists have for our publications. We are fortunate to have expert scientists as authors and editors who care strongly about what they do. We also have a group of talented, sympathetic but demanding in-house editors at the Press—Kaaren Janssen, Judy Cuddihy, and Michael Zierler—who relentlessly strive to make our books the best they can be.

John R. Inglis

years ago took a significant step forward with the signing of an agreement with a new Oxford, U.K.-based private company—Scion Publishing. Trading as Cold Spring Harbor Laboratory Press Europe, this group will have responsibility for all European sales and marketing of Cold Spring Harbor books and the fulfillment of orders through the warehouse already established in Oxford. With this initiative and the addition of sales representation in several additional European and Middle and Far East countries, our books have never been more available worldwide. There is also increasing demand to translate our books from publishers abroad, particularly in China, Taiwan, and Japan, owing to our increased international visibility at events such as the Frankfurt Book Fair and the efforts of our International Rights Manager, Anu Hanson.

In South Africa, one book has received particular attention. During 2002, 19,000 copies of the short, colorful *Staying Alive: Fighting HIV/AIDS*, by our celebrated children's book authors Fran Balkwill and Mic Rolph, were given free to young people, teachers, and community workers in three of the country's provinces. In activities coordinated in 2003 by Linzi Rabinovitz, a colleague based in Cape Town, many of the book's recipients read it, discussed it, used it in classrooms, and told us how to make it even more useful. The impetus for this book, first proposed to us by Siamon Gordon of Oxford University, is the belief that people who truly understand the nature and behavior of the AIDS virus will also realize they have the power to act to protect themselves. Thanks to the advice of the many South Africans who helped us with the book, we are now more confident that its lively drawings and uncomplicated text do convey that knowledge. With the support of a generous grant from the Bill and Melinda Gates Foundation, received in March, we will be able to distribute a much larger number of copies of an extensively revised edition of the book, translated into four more of the 11 official languages of South Africa, and create other materials to assist teachers in improving the HIV/AIDS curriculum.

We continue to use a variety of ways to promote our books nationally and internationally. A handsome catalog and two issues of our eye-catching newsletter were selectively mailed, and many thousands of scientists elected to receive e-mail alerts about new titles. Our exhibit stand was busy at all the major American scientific society meetings, as well as, this year, at the conference of the National Science Teachers Association, the International Genetics Congress in Melbourne, Australia, and the Congress of the newly formed Human Proteomics Organization. Bookstore signings were arranged for some authors, and signings at our exhibit stand were popular among conference attendees, especially Jim Watson's signing at the American Society for Cell Biology, where he was kept busy for more than 90 minutes by a long line of eager autograph seekers.

Journals

Journal publishing, which began 16 years ago with one title, is now a core competence of the Press. In 2003, the journal program grew to a total of five publications with the addition of *RNA*, the journal of The RNA Society. In the first year with Cold Spring Harbor, this eight-year-old journal benefited from significant additions to its subscription base and advertising revenue.

The journal program as whole was financially affected by the bankruptcy early in the year of a large subscription agent and the loss of more than 100 institutional subscriptions. In other respects, institutional subscriptions held steady and there was a substantial increase in the number of multisubscription licenses bought by companies and library consortia. *Genes & Development* and *Genome Research* achieved impact factors of 18.77 and 9.86, respectively, that placed them among the handful of most important journals in their fields.

These journals also received unprecedently large numbers of manuscript submissions, and since *Protein Science* and *Learning & Memory* also expanded in size, the journal staff produced over 30% more pages than in the previous year. They were assisted by technological improvements in handling digital artwork and the flow of materials for both print and on-line publication. Advertising sales in the journals improved by 12% on the previous year, assisted by the new classified advertising program Career Tracks.

During the year, we paid close attention to the momentum of the concept of "open access" in journal publishing. The phrase refers both to a principle—that scientific information should be freely available to all after publication—and to a business model, in which authors pay to publish and readers read

FINANCE

HISTORY OF THE COLD SPRING HARBOR LABORATORY ENDOWMENT

G. Morgan Browne served as the Administrative Director and, later, as Chief Financial Officer of Cold Spring Harbor Laboratory for more than 19 years. Upon his retirement in 2003, he compiled the following history of the Cold Spring Harbor Laboratory Endowment, a fund he was largely responsible for growing and maintaining.

In the early months of 2004, the Laboratory's endowment, consisting primarily of the Robertson and Cold Spring Harbor Funds, is valued at more than $225 million, and this year, it will provide nearly $7 million of financial support for the Lab's research and education programs. It is an extraordinary resource for a relatively small-sized institution. The endowment is a major source of long-term stability for the senior staff. It funds critical scientific equipment and building projects and is key to attracting promising young scientists and students from all over the world to Cold Spring Harbor. The endowment also makes it possible to opportunistically pursue unexpected and promising new ideas and programs when other sources of funding are not available. It also allows the Lab's senior leadership to sleep nights while placing major bets on breakthrough science.

But it was not always so at Cold Spring Harbor and the history of the endowment is an interesting story. Some 30 years ago in 1963, John Cairns came here from Australia to be the first Director of the Cold Spring Harbor Laboratory of Quantitative Biology (now the present CSHL) which had been newly formed to consolidate and run the operations of the laboratories previously operated at Cold Spring Harbor by the Carnegie Institution of Washington and the Long Island Biological Association (LIBA). In his first Director's Report, Cairns listed some "conspicuous deficiencies of the new Institution—namely, a deficit budget, a dilapidated plant, inadequate operating cash and a complete absence of any endowment." Although various directors at Cold Spring Harbor had been vainly seeking endowment for more than 70 years, Cairns urged that it now be given the highest priority. Several years later he was to say, "It would be fitting to be able to state that some start had been made in the quest for endowment. There is, however, no such news to report nor, even the prospect of it."

By 1968, the finances of the Lab had improved only marginally, primarily due to profitable sales of the annual volume of Symposium proceedings. James Watson was the new Director and, like Cairns, faced daunting financial problems. "There remains the fact," he wrote in his first Director's Report, "that the Lab badly needs a real benefactor, but with much love it will probably survive without one. Of course, I dream an angel will appear soon and make me free of any serious worries for at least a month." It would take another four years before there was any such help. For Jim, the good news arrived during the summer of 1972 when he and his wife Liz were in California. Edward Pulling, then Vice Chairman of the Laboratory telephoned to say that Charles Robertson, a long time resident of Lloyd Harbor, was interested in making a substantial donation. Would Jim meet with him and tell him of his future plans for the Lab?

Mr. Robertson had married Marie Hoffman in 1936. She was a member of the Hartford family that had founded the Great Atlantic and Pacific Tea Company. Charles and Marie, who died in the summer of 1972, did not believe in spreading their philanthropy around to a large number of worthy institutions. Instead, they concentrated their resources, "striving for excellence and hoping to win the big prize..." Charles Robertson had previously made a gift of $35 million to his alma mater, Princeton University,

which was subsequently used to endow the Woodrow Wilson School of Public and International Affairs. He wrote later that Cold Spring Harbor Laboratory was "right at our front door and had been a vital part of our community for over eighty years," and that it was a logical and appropriate choice.

Mr. Robertson's proposed gift was the $8 million Robertson Research Fund, to be used for the purpose of basic research. He described the Fund as the result of the many gifts made by Marie during her lifetime to the Robertson Family's Banbury Fund, which would now be the source of funding for the Research Fund. Jim Watson wrote that, "the creation of this Fund ranks as one of the most important events in our Lab's history."

The Robertson Research Fund Inc.

At the end of 1972, Mr. Robertson established a tax-exempt 501 (c) (3) corporation, The Robertson Research Fund Inc., which was "at all times thereafter to be operated, exclusively for the benefit of ... and to carry out the purposes of the Cold Spring Harbor Laboratory." There were to be nine directors, five representing the Laboratory and four representing members or agents of the Robertson family. Mr. Robertson would later donate his Lloyd Harbor Estate to the Lab as a conference center (now our Banbury Center). He also established the Robertson Maintenance Fund, an additional $1.5 million endowment, to cover the costs of maintaining the buildings and grounds of the property in perpetuity.

Mr. Robertson had been a long-time admirer of Walter H. Page II, who was then President of J.P. Morgan & Co. and had been Chairman and a member of the Long Island Biological Association, until the pressure of Bank business took him to London. Now at the urging of Mr. Robertson, who greatly admired his financial acumen, Mr. Page agreed to again join the Lab's Board of Trustees, serving later as its Chairman from 1980 to 1986.

The Robertson Research and Maintenance Funds

Discussions with lawyers and the Internal Revenue Service took more than two years, and it was not until June of 1975 that the Board of Trustees was able to pass a resolution formally accepting the Robertson Funds as the Lab's first endowment. In the meantime, Mr. Robertson's gift had been invested, and $751,000 of income was distributed during the 1973–1975 period in support of the Lab. The first investment advisor for the Fund was the New York City firm of John W. Bristol & Co. An investment proposal from Bruce D. Bent, a Vice President of Bristol & Co., proposed a 50/50 ratio of debt to equity, yielding interest income of 8% from the debt portion in AAA-rated obligations and dividend income of 7% from a variety of blue chip stocks. How times have changed! Many of these equities were members of the then so-called "nifty fifty"—companies that supposedly could be counted on to grow by 10–20% every year and raise their dividends ad infinitum. Some of them lost as much as 90% of their value during 1974. From a market value at the end of 1973 of $8.3 million, the Robertson gift plummeted to $6.3 million by the end of 1974.

Investment Management Advice

It could have been much worse, but Charles Robertson had never been a fan of the "nifty fifty" concept and a 50% fixed-income allocation saved the portfolio from more severe damage. As 1975 ended, it had recovered to $7.3 million. The Investment Committee for the Robertson Funds, then consisting of Mr. Robertson, James Watson, Angus McIntyre, Clarence Galston, Townsend Knight, and William R. Udry, initiated a search for alternative investment management. No less than 11 firms were interviewed, and thanks in particular to the hard work of Bud Galston and Angus McIntyre, the Committee chose the Bala Cynwyd, Pennsylvania firm of Miller Anderson & Sherrerd. Mr. Robertson marked the occasion with a note to each of the Committee members saying in part, "Only the future will tell whether you

have chosen wisely and well with Miller Anderson and Sherrerd, but I'll certainly doff my Bonalino to you for having tried and hard. My deep appreciation to each one of you for your dedication to duty."

Miller Anderson & Sherrerd

In January of 1976, A. Morris Williams, then a 35-year-old account executive at Miller Anderson, assumed responsibility for the Robertson Funds account. Morris was hardly a newcomer to the investment business. After earning both a B.A. and M.A. at Duke University, he attended the Stanford Investment Management Program and the Harvard University Investment Management Workshop. It was the start of a fortuitous relationship for the Lab with Miller Anderson. From the close of 1975 to 1996, when the firm was acquired by Morgan Stanley Dean Witter, the Research Fund appreciated from $7.3 million to $62.1 million and the Maintenance Fund appreciated from $1.5 million to $10 million. The only additions to the Funds were reinvested income and $643,000 of remaining proceeds from a Trust that Mr. Robertson had established on behalf of his second wife, Jane Cain Robertson. This Trust reverted to the Research Fund upon her death in Florida in 1993. The $63.3 million increase in the total value of the Funds was *after* distributions to the Laboratory totaling just over $20 million. Of these 20 years, only 4 years showed a decline in the value of the Funds, and if calculated before the annual distributions of income, there was only one such year, 1977, and that by just 1.2%. When Morris Williams retired from Miller Anderson in 1991, he made a gift on that occasion to the Laboratory of $100,000 in support of our then new neuroscience program.

Credit for the 21-year good market performance of the Robertson Funds should properly be shared, and not least with the mostly strong equity markets of the period. The Robertson Funds Investment Committee, later replaced by the Laboratory's Finance and Investment Committee, also had an important role. The members met regularly with Morris Williams for luncheon three or four times a year, initially at the Princeton Club and later at the Sky Club at the Pan Am Building (now Met Life). Attendance at these luncheons was nearly always excellent, perhaps also prompted by the members' personal interest in the ready availability of good investment advice.

The Committee established basic policy, namely, that the Funds were to be managed conservatively both for income and for sufficient growth to keep pace with inflation. There was to be a maximum 65% and a minimum of 40% equity allocation, with the balance to be in fixed income. Miller Anderson was responsible for changes in asset allocation, which were generally reviewed by the Committee with a minimum of second-guessing. Investments were to include a balanced mix of large- and small-cap equities with a growth bias and high-quality domestic-only fixed-income securities. Morris Williams and the Miller Anderson investment analysts made the selections of individual stocks and bonds, with at times lively advice from Committee members. At one memorable meeting, Jim Watson objected vociferously to an intended investment in McDonalds Corporation, stating that he had recently lunched at Burger King and it was much better than McDonalds. McDonalds became part of the portfolio anyway, and not too long afterward, Jim stopped coming to the meetings.

David L. Luke III, who as Treasurer of the Laboratory from 1986 became Chairman of the Committee, opposed investments in foreign equities on the grounds that large American companies had more than sufficient foreign exposure from their own overseas business activities. A small investment in an international fund recommended by Miller Anderson was quickly liquidated after a few months of down performance. Despite its excellent growth record, Westvaco Corporation, of which Mr. Luke was then Chairman, never appeared in the portfolio—much to the wonderment of some Committee members. When Morris was complimented on the performance of the portfolio, he never tired of letting the Committee know how much additional appreciation would have resulted had he been permitted to buy Philip Morris shares. The Committee felt strongly that it would be inappropriate for an Institution aiming to cure cancer to be investing in tobacco companies.

The Lab's very conservative drawdown policy was certainly a very major factor in the growth of the Funds. At a time when most colleges and universities were drawing 5% or 6% annually from their endowments, the Lab disciplined itself to draw just 4%, based on a three-year moving average of year-

end market values. Since the three-year moving average in a generally up market was almost always lower than the most recent year, this policy resulted in an even lower drawdown than 4%, more often in the area of 3.5%. All dividends, interest income and capital gains, net of expenses, and distributions were reinvested in the Funds.

The Second Century Capital Campaign and the Cold Spring Harbor Fund

In 1986, the year after I joined the Laboratory as Administrative Director, the Robertson Funds continued to be the primary internal source of funding for Lab science, but they were no longer to be the sole endowment. That year, the Lab's first major capital fund-raising campaign, the Second Century Fund, was launched under the leadership of David Luke. The initial goal for the private phase of the campaign was $30 million. In April of 1989, with $28 million pledged or in hand from Trustees, private foundations, and a few corporations, the goal was raised to $44 million and the public phase began. The campaign included a mix of money for capital construction, primarily the Beckman Neuroscience Center, and both restricted and unrestricted endowment for faculty chairs, fellowships, and programs. At its conclusion in 1992, the Second Century Fund campaign had raised a grand total of $51 million.

As the new monies flowed in during 1986 and 1987, the Lab's Board of Trustees established the Cold Spring Harbor Fund, which was to hold the new, and one previously received, endowment-type donations. The new Cold Spring Harbor Fund and the Robertson Funds would thereafter constitute the Lab's overall endowment. They would be managed jointly and be subject to the same general policies concerning investment management and annual distributions.

The Doubleday Professorship of Cancer Research

The Laboratory's first endowed faculty Chair—the Doubleday Professorship of Cancer Research—was the initial component of the Cold Spring Harbor Fund. It resulted from a gift actually received in 1984 of 500 shares of Doubleday & Co. from the Russell and Janet Doubleday Fund, then thought to be worth a few hundred thousand dollars. To the amazement of all concerned, following the sale of Doubleday & Co. to the publishing giant, Bertelsmann, Inc., the Lab received $2.1 million for the shares in 1987 and used $1.5 million of this amount to establish the Chair. The Lab's Board of Trustees had determined that named endowed chairs should be funded at this level. Mike Wigler has been the Doubleday Professor from the inception of the Chair. The remainder was used to purchase a house, now known as Doubleday, that abutted Lab property.

The Grace Director's Chair

The Oliver and Lorraine Grace Director's Chair followed soon after the same year. The Grace Chair was established by a $1.5 million series of gifts from the Helen Coley Nauts & Oliver R. Grace Endowed Chairs at the Cancer Research Institute and from Oliver R. Grace personally. Mr. Grace was Chairman of the Cancer Research Institute at the time. Dr. Watson was the first recipient of the Chair and has held it ever since. Currently, the Doubleday and Grace Chairs are each valued at more than $4 million.

The 20th Century Undesignated Fund

During the first year of the Cold Spring Harbor Fund, the 20th Century Undesignated Fund was established as one of its components. This Fund was made up of a remarkable number of unrestricted individual donations from Laboratory and LIBA Trustees and from employees and friends, which from 1987 to 1998, amounted to $4.9 million in total, with the great majority received in the earlier years. These

unrestricted gifts have been particularly valuable to the Lab over the years in enabling the Trustees to opportunistically purchase properties adjoining the Lab, which have become available from time to time. One house on Ridge Road had been seized by a federal marshal from an incarcerated drug dealer and was being sold at auction. This was to be the site of Ballybung, Jim Watson's residence, who donated the monies to the Laboratory for its construction. The neighboring Chernick House, now Olmsted House, where Scott Lowe, Associate Director of our Cancer Center, and his family now live, was purchased by the Fund. It also helped acquire the Kelman House (now the Townsend Knight House), which is home for the first-year graduate students of the Watson School, as well as the Galehouse adjacent to Airslie. The Undesignated Fund was also used to help scientists purchase local homes and for various investment management and development expenses. Just recently, the donor of a new endowed Chair conditioned the grant on the Lab's participation in the amount of $250,000. Once again these unrestricted moneys were called on for an important opportunity. Thanks to market appreciation and reinvested income, the Fund today stands at more than $3.5 million without counting the present value of the real estate purchased by the Lab.

The Cold Spring Harbor Fund continued to grow rapidly with new additions during 1988 and 1989. The Charles Robertson Neuroscience Chair was created with an initial gift of $300,000 from the Banbury Fund under the sponsorship of its Trustees—the Ernsts, Meiers, and Robertsons. Charles Robertson was the father of Katherine Ernst, Anne Meier, and Bill Robertson. A series of annual additional gifts and market appreciation soon brought the funding to beyond the $1.5 million target. Another addition was the Posy White Memorial Fund, established by Elinor Montgomery in memory of her mother, Posy White, who had served on the LIBA and Laboratory Boards for some 25 years. Elinor was a Trustee of the Lab from 1988 until her death in 1992. The Senior Staff Research Fellowships were created in 1990 by the Gladys and Roland Harriman Foundation with a two-for-one challenge grant that was soon satisfied by individual donations.

In 1992, the W.M. Keck Chair was funded by a $1.5 million grant from the W.M. Keck Foundation for the support of research in the Keck Structural Biology Laboratory, which was established in conjunction with a previous grant for construction of the Beckman Neuroscience Center. Also that year, the Harrison Chair for the Study of Molecular Neuroscience was established from the estate of Maxine Harrison in memory of her mother, Ally Davis Harrison. Ms. Harrison served as a foreign news correspondent during World War II for an English language newspaper in the Philippines. She lived the final years of her life as a resident at the Piping Rock Club in Locust Valley and was a frequent visitor to the Lab's Carnegie Library where she enjoyed reading and researching newspaper and other archives.

In 1993, the William J. Matheson Professorship was created based on a $500,000 challenge grant from the Fort Hill Foundation. The Lab raised the additional $1,000,000 needed for full $1.5 million funding from the Mary C. Turner Charitable Trust, other outside donations, and internal funds, as well as from market appreciation. This was the Lab's sixth fully endowed faculty chair. The Matheson Professorship was intended as a tribute to the memory of William J. Matheson, who was an important member of the local community and in 1905 was President of the Bio-Lab's Board of Managers. The Bio-Lab was one of the predecessors of the present Cold Spring Harbor Laboratory. The Chair is currently held by Bruce Stillman.

Tax-exempt Financing and the 20th Century New Endowment Fund

Another important early component of the Cold Spring Harbor Fund was the 20th Century New Endowment Fund, which was made possible by the availability to the Lab of tax-exempt financing for capital construction. In the mid-1980s, the Lab's need for a modernized plant, facilities, and infrastructure had become increasingly urgent. With much trepidation at the time, it was decided to take advantage of the availability of very low-cost debt financing through the sale of daily-rated tax-exempt bonds authorized by the Nassau and Suffolk County Industrial Development Agencies, backed by a letter of credit from the Morgan Guaranty Trust Company. The interest rate has been remarkably low, about 3% over the life of the bonds, about 1% recently. The primary purchasers have been New York

State tax-exempt money market funds. The initial issuance in 1984 of $8 million of Civic Facility Revenue Bonds helped speed additions to James and Demerec laboratories, the construction of Grace Auditorium, modernization of Blackford Hall, and sorely needed additional parking areas. In 1988, an additional $20 million issuance of similar bonds provided us with great flexibility in managing the cash flow and timing of a then very large construction program. More recently in 1998, a third issuance of $42 million refinanced $27 million of the previous debt and provided $15.2 million toward construction of the Woodbury Cancer Genome Research Center. Today, there are $45.2 million of bonds outstanding—$3 million maturing in 2023 and $42.2 million in 2034. A rating of A1+ was obtained from Standard & Poor's, made possible in part by the Lab's then nine consecutive years of operating surpluses and positive cash flow. This enabled the bonds to be backed by a liquidity facility from Morgan Guaranty Bank at half the cost of the previous letter of credit.

Early in the Second Century Fund campaign, it became clear that some donors preferred having their gifts used to endow research projects, rather than directly finance new buildings. By funding construction costs with tax-exempt bonds, the Lab could add such gifts to the Cold Spring Harbor Fund as endowment, and so the 20th Century New Endowment Fund was born. The Fund was segregated according to the interests of the donors as to cancer, neuroscience, education, and infrastructure. The Board of Trustees has felt strongly about maintaining the integrity of the Lab's finances and directed that these funds be managed very conservatively so as to provide the greatest possible flexibility. The Fund is therefore invested in very short-term high-quality fixed-income securities supervised by the U.S. Trust Company. The Fund has increased to approximately $32 million after reimbursing the Lab for bond interest and associated expenses and provides strong support for our balance sheet. The Howard Hughes Medical Institute, in arranging financing for their new Janelia Farm Research Campus, due to open in Washington D.C. in 2006, used the Laboratory's most recent tax-exempt financing as a model, and Morgan Guaranty Bank has become by far the largest underwriter of tax-exempt financing for 501(c)(3) institutions such as Cold Spring Harbor Laboratory.

The Science Fund and the Quest Fund

Two other specialized funds that are components of the Cold Spring Harbor Fund are worthy of special mention. The basic research and teaching at Cold Spring Harbor Laboratory helped make possible the birth of the biotechnology industry, and since the early 1990s, venture capitalists and others interested in starting biotech companies were much interested in the intellectual property developed and owned by the Lab. The intellectual property could be licensed to the new start-up companies in return for equity and the promise of future royalties. So it was that our Board of Trustees in 1992 established the Science Fund and designated it as solely for the future support of science. All equity and royalties received from the Lab's technology transfer activities have been accumulated in this Fund. In this manner, all financial gains from Laboratory research financed by federal and other grants are recycled back into future science. In the early years, the various equity holdings were carried at nominal valuations, and the Fund grew slowly to $490,000 at the end of 1996. Thereafter, many of the equities became publicly traded. John Phelan, Chairman of the Lab's Commercial Relations Committee, led a subcommittee that authorized the sale of some portions of the Lab's holdings, with the proceeds henceforth to be managed by our investment managers. Included in the Lab holdings today are positions in public companies such as Exelexis (Genomica), Geron, Icos, Pharmacopeia, Tularik, and the European company GPC Biotech (Mitotix), as well as approximately six still-private companies. By year-end 1997, 1998, 1999, and 2000, the Science Fund was valued at approximately $4.8, $6.8, $15.9, and $30.2 million, respectively. The comparable values at the end of the 2001 and 2002 bear market years were much reduced at $26.7 and $18 million, respectively. The Fund has now recovered to near $25 million—$7 million of this amount representing public and nominally valued private biotech holdings and $17 million representing proceeds of past sales and royalty income—all of which is now invested in conventional investments managed by the Lab's investment managers.

The Science Fund was also established with the hope that it might one day replace some portion of

a large royalty income, which the Lab had been receiving since 1991 from the Axel-Wigler patent issued to Columbia University. This patent was the basis for manufacturing many early genetically engineered biotech drugs and was to expire June 30 of 1999. Some additional income continued to be received through 2001, and over the life of the patent, the Lab received a total of $24,614,000. Although the Science Fund cannot fully replace such a sum, it has already far exceeded our expectations.

The second specialized fund—the Quest Fund—was established by the Lab's Executive Committee in December 1999 and designated for the support of both science and education programs at the Lab. This Fund consisted of equity ownership in SciQuest Corporation—a start-up "B to B" commerce Internet company specializing in scientific supplies and equipment—that the Lab received from the sale of the Cold Spring Harbor Laboratory Press "Source Book" (catalog) and BioSupplyNet (on-line version) to SciQuest in 1998. In 1999, SciQuest had a successful IPO, and by March 31 of that year, our shares were valued at $4.7 million. All of the Lab's shares were restricted from sale for 6 months under the terms of the underwriting agreement. Only when SciQuest freed 10% of all holdings in an attempt to slow the then meteoric rise of the stock was the Lab able to sell $1.6 million of shares. When the balance of the shares were freed from restriction, the price had declined from about $90 to $10 and later to less than $2. It is entertaining to dream of "what might have been"—at one point, the Lab's restricted position was worth nearly $25 million. Last year all the remaining shares were sold back to SciQuest in return for the rights to the Source Book and BioSupply Net, which it is believed may have a brighter future in the regular business of the Press. The Quest Fund currently has a value of about $2 million, representing the now conventionally managed proceeds of the early sales.

The Science and Quest Funds have allowed the Lab to participate in venture-capital-type biotech and Internet holdings on a meaningful and very profitable basis without investing a penny. Recently, in 2001, the Investment Committee authorized investment of up to $1 million in a new venture capital fund of funds organized by senior members of the finance and investment department at Massachusetts Institute of Technology. To date, only about a third of the money has been invested, and it is too early on the J-shaped curve typical of such investments to judge the outcome.

More Additions to the Cold Spring Harbor Fund

The Cold Spring Harbor Fund continued to grow with substantial new additions throughout the 1990s. The Undergraduate Research Program (URP) Fund is a grouping of fellowships established by good friends of the Lab such as the Libby, Olney, and Darrell families, and $100,000 donations each from the Estate of Joan Read and from the Garfield Foundation of Princeton, New Jersey. Noted diva Frederica Von Stade established a named Internship with the proceeds of two marvelous concerts at the Lab, the first entitled "Mozart to Gershwin" in 1990 at the Lab's Centennial celebration, and a second in 1995 that was a wonderful blend of ethnic folk, bluesy jazz, and ragtime. Likewise in 1996, the renowned concert pianist Emanuel Ax performed brilliantly in Grace Auditorium, and the proceeds were used for an Ax Fellowship. The URP Fund endowment was completed by a $1 million grant from the Burroughs Welcome Company later the same year, thanks to the good relations established there by Jill Clark, then a very talented member of our Development Department. The URP Fund now totals over $3 million.

The Marjorie Matheson Neuroscience Fund

The 1995–1996 period was highlighted by extraordinary gifts from the Marjorie A. and William L. Matheson Charitable Trust, which funded the $3 million Marjorie Matheson Neuroscience Fund. The Mathesons were extremely generous and made the gift at the perfect time. The Lab's neuroscience program was just then gaining momentum, and the Matheson's vote of confidence helped enormously. Today, the Lab neuroscience program is among the best in the world. The Marjorie Matheson Neuroscience Fund is now valued at more than $6 million.

During this period, Jim Watson established the Gavin Borden Fund to honor and remember his

good friend and publisher of *The Molecular Biology of the Cell.* Each year, it funds a lecture and dinner at the Lab featuring a prominent visiting scientist. The Nat Sternberg Thesis Prize Fund was established to honor the life and accomplishments of Nat Sternberg, a scientist who worked for many years at the DuPont Merck Pharmaceutical Company on many aspects of phage biology. This endowment was funded by DuPont and more than 100 individuals, families, foundations, and companies. Each year, it funds a cash award to a student with the year's outstanding Ph.D. thesis in the field of prokaryotic molecular genetics at the annual Molecular Genetics of Bacteria and Phages Meeting held every other year at Cold Spring Harbor.

The Watson School Fund

In November of 1998, a long-standing initiative came to fruition when the New York State Board of Regents accredited Cold Spring Harbor Laboratory as a Ph.D.-granting institution. Early the next year, our new graduate school, the Watson School of Biological Sciences, was born. In addition to an innovative curriculum, the new school would have two distinctive feature: Ph.D. candidates would be expected to graduate in four years and each would be fully endowed. To accomplish the latter, the Board determined that a $32 million endowment would be needed. It was to include a $5 million Founders Endowment, individual $1 million named fellowships, a Dean's Chair, endowed core courses of study, lectureships for Watson School and visiting faculty, and other special-purpose funds.

In November of 1998, David Luke retired from his 12-year tenure as Chairman of the Board and was named an Honorary Trustee. William R. Miller was chosen as his successor. When David Luke then agreed to chair the campaign for the Graduate School, all of us knew it would be a great success. The private phase of the campaign was conducted in the closing months of 1998 and early 1999. As one would expect with any endeavor led by David Luke, the effort was intense and progress was rapid and gratifying. In this short time, nearly $12 million was pledged or in hand.

The Founders Endowment, the Dean's Chair, and Six Fellowships

In July, Marjorie Matheson made another magnificent gift, this time funding the Founders Endowment in honor of her parents George A. and Marjorie H. Anderson. This Endowment would cover many of the School's start-up expenses and also provide for five named fellowships. Through the efforts of Leon and Cynthia Polsky, the Dean's Chair was established with gifts of $1,175,000 from the Lita Annenberg Hazen Charitable Trust and of $500,000 from the Annenberg Foundation. Named student fellowships were established by past and present Lab Trustees: Bill Miller, David Luke, David Koch, Martha Gerry (through the William Stamps Farish Fund), Leslie Quick, and Bob Lindsay. Alan Goldberg, a close friend of Bob Lindsay, also pledged a Fellowship.

David Luke was honored at a dinner on April 23, 1999 at the Piping Rock Club in Locust Valley in appreciation of his years of service, and the occasion also served as a launching platform for the public phase of the campaign. Vartan Gregorian, President of the Carnegie Corporation of New York and past President of Brown University, was the keynote speaker.

During the public phase, Mr. And Mrs. Leslie C. Quick, Jr. established the Fund for Innovative Graduate Education. Kurt Engelhorn, who had come to know the Lab well through years of attendance at annual Banbury Center corporate conferences, established five fellowships for European students to be known as Engelhorn Scholars through the European Foundation for the Advancement of Medicine. The Arnold O. and Mabel M. Beckman Foundation provided $1 million for student and core course support. Bill Miller and David Deming devoted much effort to approaching the pharmaceutical industry for endowment. Bristol-Myers Squibb Company, of which Bill Miller had been Vice Chairman, responded favorably with a $1 million named fellowship and Pfizer funded a named lectureship. Otherwise, it was a hard sell due to then poor economic conditions and policies within many companies prohibiting donations for endowments.

Named fellowships were provided by the Charles Dana Foundation, the Florence Gould Foundation, and the Hearst Foundation. The Estate of Elisabeth (Betty) Sloan Livingston, a very good friend of the Lab and an enthusiast of the new Graduate School, provided two $1 million named fellowships. Four Faculty and Visiting Lectureships were funded by Edward and Martha Gerry. The Rathman Family Foundation donated a $300,000 Lectureship. Others were donated by the Klingenstein Foundation and by John and Rita Cleary, George and Lucy Cutting, Mary Lindsay, Henry Harris, and the Ziering Family. Norris Darrell funded the Core Course in Exposition and Ethics. There were additional generous gifts from Lazard Frere & Co., the Koshland Foundation, Mike Wigler, Karen and Mark Zoller, and many others. In the final year of the campaign, the $1 million Watson Fund for Innovative Education was assembled with the help of a $350,000 challenge grant from the Gladys and Roland Harriman Foundation and $100,000 from a fund in honor of David Pall (past Lab Trustee and founder of the Pall Corporation), as well as from countless other generous gifts.

The Watson School Gala: Emanuel Ax, Midori, and Yo-Yo Ma

Although not strictly for endowment, the Watson School Gala held in Grace Auditorium on October 5, 1999 must be mentioned for its role in creating a tone of unmistakable quality and excitement that permeated the entire fund-raising effort for the Watson School. This memorable event, masterfully chaired by Trustee Lola Grace, was made possible through the efforts of Trustee Mark Ptashne who persuaded three of the world's foremost classical musicians—pianist Emanuel Ax, violinist Midori, and cellist Yo-Yo Ma—to perform a joint program of Mendelsohn. More than $750,000 was raised that evening and subsequently used toward the renovation of the Knight House, first-year residence for the students of the Watson School.

The Robertson and Cold Spring Harbor Funds Add Investment Managers

Although the Robertson Funds had continued to perform well after Morris William's retirement in 1991, the Finance and Investment Committee by 1996 was not pleased that the account was now being invested on a commingled basis with other accounts at Miller Anderson, rather than in individually selected securities as in the past. Moreover, the Robertson Funds had grown substantially, and with the addition of the new moneys of the Cold Spring Harbor Fund, the Investment and Finance Committee decided that it would be prudent to have more than a single investment manager. Under the leadership of John Reese, then Lab Treasurer and Chairman of the Committee, a search was instituted to select two or more additional managers with an emphasis on large- and small-cap growth stocks. Miller Anderson would be retained for their deep-value equity product and also as fixed-income manager. With regard to the latter, we were most fortunate in having the individual attention of the very capable Ellen Harvey, a relative of David Luke, as our fixed-income manager. Not only did Ellen deliver first-rate investment performance, but she was also capable of making understandable and interesting presentations to the Committee on the intricacies of derivatives, swaps, and other new fixed-income products then coming into vogue.

Vanguard PRIMECAP and Essex Investment Management

The New Jersey investment management consulting firm of Hamilton & Co. was engaged following interviews with many of the other important names in the field. Hamilton was to conduct the search and make recommendations from among the highest-rated managers in their universe of managers. Hamilton was also to provide monthly performance reports comparing managers with their peers and keep the Committee current on any important style or personnel changes among the managers. An interview process extending over several months ended with the selection of the San-Francisco-based

PRIMECAP Fund of the Vanguard Group and Boston-based Essex Investment Management. Only then was it discovered that PRIMECAP had just recently closed its doors to new investors as a result of recent very rapid growth in the size of the Fund driven by its outstanding performance, which the principals feared would hinder future results. Fortunately, the Robertson Family's Banbury Fund had been a long-time investor in PRIMECAP, and Bill Robertson was able to intercede and persuade PRIMECAP to be receptive to accepting funds from the Lab. PRIMECAP's success was built on selecting high-quality medium- to large-cap growth companies and staying with them long term or until there was a major change in outlook or management strategy. Essex Investment Management had become successful by identifying new high-tech companies with exceptional promise. Joe McNay, a senior Essex partner, agreed to personally manage the Lab's accounts. His biannual presentations of new technology business models were invariably the highlight of Finance and Investment Committee meetings. Joe not only was very good at stock selection, but made effective use of taking only small initial positions and adding to them if they performed well, and quickly liquidating them while they were small if they performed poorly.

With new investment managers in place, the Robertson and Cold Spring Harbor Funds participated happily in the four-year Alan-Greenspan-dubbed "irrational exuberance" bull market which was to finally culminate in 1999. That year, Essex returned 111%, exceeding 99% of all comparable investment managers. PRIMECAP returned an impressive 42%. Only the performance of the Miller Anderson value fund was disappointing. The firm seemed to have lost much of its identity and many of its top people in the merger with Morgan Stanley Dean Witter. On the advice of Hamilton & Co., the Committee chose to replace Miller Anderson with the highly regarded Diversified Value Portfolio managed by Dave Williams at the U.S. Trust Company. Although only having the Lab's money for less than six months, Dave was able to comfortably outperform the Standard and Poor's 500 Index for the year. In the early months of 2000, the value of the Lab's overall endowment reached an all time high of nearly $240 million. The Robertson Funds were valued at $110 million and the Cold Spring Harbor Fund at $130 million. There was to follow a brutal almost three-year bear market. From March 10, 2000 and September 14, 2000, respectively, the NASDAQ and Standard & Poor's 500 Indexes declined by 78% and 49% until the market reached bottom on October 9, 2002.

As in 1973, the Lab's portfolio was saved from severe damage by a 50% fixed-income allocation through much of the period. Nevertheless, the endowment suffered its first ever consecutive years of decline. Essex stumbled badly in not liquidating in time the portfolio's volatile high-tech positions. PRIMECAP held to its positions in high-quality growth companies, and many of these in the telecommunications, semiconductor, and airline industries were ravaged by economic conditions and world events. At the bottom of the bear market in October of 2002, the Lab's overall endowment had declined to approximately $181 million.

The bear market experience has provided a good lesson in the importance of having in place an asset allocation policy that forces the reduction of equity when bull markets drive values above targeted levels and forces purchases when bear markets have the opposite effect. It is unrealistic to expect Investment Committees to effectively do asset allocation when meeting only several times a year or more frequently by telephone. Too late we also recognized that the Lab's equity managers may have been too highly correlated for adequate diversification. An asset allocation and investment strategy review is under way. Significantly, the Investment Committee has recently contracted for investment management consultative services from Merrill Lynch and authorized an initial investment in a very promising high-quality new "fund of funds" hedge fund—Blue Orchid Capital.

A Solid Future

This story of the Lab's endowment ends with the very good news that its value is again near an all time high. Dill Ayres, now Chief Operating Officer, notes that the Investment Committee, chaired by Lab Treasurer Lola Grace, had the courage in March of 2003 to recommit to equities and increased that asset allocation to 60%. Our investment managers all handsomely outperformed the Standard and

Poor's 500 Index, allowing our equity portfolio to appreciate by 39% for the calendar year as compared with 28% for the Standard and Poor's. The fixed-income portfolio now managed by Pimco Investment Management also did well, increasing 5%, somewhat ahead of the benchmarks.

In addition, after a gap of several years, there was an important addition. The Lab's seventh endowed professorship, the St. Giles Chair of Neuroscience Research, was established and will be fully funded over the next several years.

In the years ahead, the need for endowment can only continue to grow, and probably at an accelerating pace. Our ambitious program of new science buildings, a campus for the Watson School, and requisite infrastructure all carry with them an ongoing burden of overhead in addition to their construction cost. Overhead associated with science buildings and their infrastructure can be at least partially defrayed by indirect cost recoveries on government grants. But this is not the case with the Watson School for which a new funding source must be found.

During the past year, there has been much talk at meetings with a number of investment consultants about reduced expectations for market returns over the next decade, and the likelihood that even a 4% drawdown from endowment may be too high if principal is to be fully preserved on an inflation-adjusted basis. The recent rally in the equity markets has reduced some of the gloom, but this is a subject to take very seriously.

I fear the end result must be still more work for the Laboratory's Board of Trustees in ensuring that the past remarkable pace of new additions to the Cold Spring Harbor Laboratory Endowment continues, and for the Administration in making sure that the Endowment continues to be managed carefully and well. Only then will it be certain that this invaluable resource continues to fully serve the needs of this great Institution.

G. Morgan Browne
March, 2004

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2003
With comparative financial information for the year ended December 31, 2002

Assets:	*2003*	*2002*
Cash and cash equivalents	$ 28,971,185	15,998,950
Accounts receivable:		
Publications	1,257,221	1,507,910
Other	1,332,279	621,788
Grants receivable	7,068,387	5,155,499
Contributions receivable, net	11,452,991	4,485,178
Publications inventory	2,206,640	2,214,499
Prepaid expenses and other assets	1,566,991	1,630,366
Investments	198,696,165	174,146,179
Investment in employee residences	4,774,834	4,347,627
Restricted use assets	2,240,000	2,240,000
Land, buildings, and equipment, net	109,253,408	108,416,078
Total assets	$ 368,820,101	320,764,074
Liabilities and net assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 5,505,705	4,347,089
Notes payable	140,958	170,119
Bonds payable	45,200,000	45,200,000
Deferred revenue	4,067,960	3,648,453
Total liabilities	54,914,623	53,365,661
Net assets:		
Unrestricted	178,415,767	154,939,682
Temporarily restricted	14,223,309	10,132,940
Permanently restricted	121,266,402	102,325,791
Total net assets	313,905,478	267,398,413
Total liabilities and net assets	$ 368,820,101	320,764,074

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2003
With comparative totals for the year ended December 31, 2002

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2003 Total*	*2002 Total*
Revenue and other support:					
Public support (contributions and nongovernment grant awards)	$ 13,391,893	14,223,309	4,822,932	32,438,134	25,057,008
Government grant awards	24,231,989	–	–	24,231,989	22,680,109
Indirect cost allowances	18,000,004	–	–	18,000,004	15,073,780
Program fees	3,134,443	–	–	3,134,443	2,910,787
Publications sales	10,052,963	–	–	10,052,963	9,050,738
Dining services	2,927,668	–	–	2,927,668	2,850,201
Rooms and apartments	2,347,090	–	–	2,347,090	2,222,483
Royalty and licensing fees	869,826	–	–	869,826	1,173,659
Investment income (interest and dividends)	4,141,495	–	–	4,141,495	5,515,942
Miscellaneous	616,755	–	–	616,755	583,033
Total revenue	79,714,126	14,223,309	4,822,932	98,760,367	87,117,740
Net assets released from restrictions	10,132,940	(10,132,940)	–	–	–
Total revenue and other support	89,847,066	4,090,369	4,822,932	98,760,367	87,117,740
Expenses:					
Research	45,619,807	–	–	45,619,807	42,240,020
Educational programs	12,917,185	–	–	12,917,185	12,105,086
Publications	10,631,334	–	–	10,631,334	9,345,581
Banbury Center conferences	1,136,159	–	–	1,136,159	1,141,308
Dolan DNA Learning Center programs	1,809,174	–	–	1,809,174	2,368,850
Watson School of Biological Sciences programs	2,029,275	–	–	2,029,275	1,648,168
General and administrative	11,976,718	–	–	11,976,718	10,562,264
Dining services	4,262,515	–	–	4,262,515	4,146,716
Total expenses	90,382,167	–	–	90,382,167	83,557,993
(Deficiency) excess of revenue and other support over expenses	(535,101)	4,090,369	4,822,932	8,378,200	3,559,747
Other changes in net assets:					
Net appreciation (depreciation) in fair value of investments	24,011,186	–	14,117,679	38,128,865	(27,887,903)
Increase (decrease) in net assets	23,476,085	4,090,369	18,940,611	46,507,065	(24,328,156)
Net assets at beginning of year	154,939,682	10,132,940	102,325,791	267,398,413	291,726,569
Net assets at end of year	$ 178,415,767	14,223,309	121,266,402	313,905,478	267,398,413

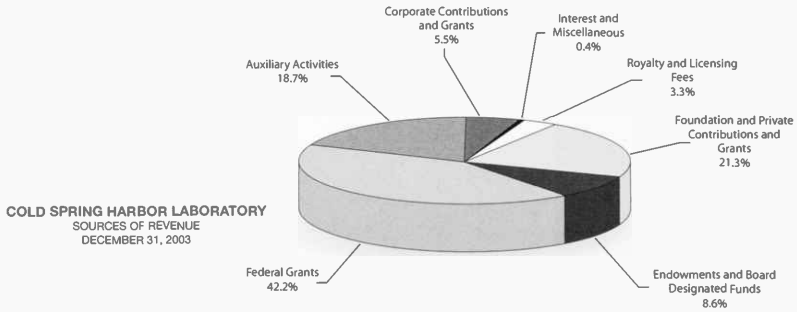
CONSOLIDATED STATEMENTS OF CASH FLOWS
Year ended December 31, 2003
With comparative financial information for the year ended December 31, 2002

	2003	2002
Cash flows from operating activities:		
Increase (decrease) in net assets	$ 46,507,065	(24,328,156)
Adjustments to reconcile increase (decrease) in net assets to net cash (used for) provided by operating activities:		
Depreciation and amortization	5,458,719	5,165,482
Net (appreciation) depreciation in fair value of investments	(37,868,190)	27,594,426
Contributions restricted for long-term investment	(13,460,462)	(5,020,793)
Restricted use asset	–	(840,000)
Changes in assets and liabilities:		
(Increase) decrease in accounts receivable	(459,802)	1,337,585
(Increase) decrease in grants receivable	(1,912,888)	39,125
(Increase) decrease in contributions receivable	(875,375)	1,754,273
Decrease (increase) in publications inventory	7,859	(187,369)
Decrease in prepaid expenses and other assets	63,375	806,071
Increase (decrease) in accounts payable and accrued expenses	1,158,616	(1,581,205)
Increase in deferred revenue	419,507	1,033,081
Net cash (used for) provided by operating activities	(961,576)	5,772,520
Cash flows from investing activities:		
Capital expenditures	(6,296,049)	(5,476,620)
Proceeds from sales and maturities of investments	135,393,757	185,039,464
Purchases of investments	(122,075,553)	(200,832,292)
Net change in investment in employee residences	(427,207)	148,147
Net cash provided by (used for) investing activities	6,594,948	(21,121,301)
Cash flows from financing activities:		
Permanently restricted contributions	4,822,932	3,605,790
Contributions restricted for investment in land, buildings, and equipment	8,637,530	1,415,003
(Increase) decrease in contributions receivable	(6,092,438)	441,643
Repayment of notes payable	(29,161)	(32,035)
Net cash provided by financing activities	7,338,863	5,430,401
Net increase (decrease) in cash and cash equivalents	12,972,235	(9,918,380)
Cash and cash equivalents at beginning of year	15,998,950	25,917,330
Cash and cash equivalents at end of year	$28,971,185	15,998,950
Supplemental disclosures:		
Interest paid	$ 1,014,956	1,132,279
Noncash investing and financing activities:		
Contributed property	$ –	840,000

COMPARATIVE OPERATING HISTORY 1999–2003
(Dollars in Thousands)

	1999	2000	2001	2002	2003
Revenue:					
Main Lab:					
Grants and contracts	$ 27,179	30,345	34,716	37,872	41,749
Indirect cost allowances	11,207	12,718	14,134	14,987	17,869
Other	9,426	10,618	12,528	10,918	10,524
CSHL Press	6,400	8,684	9,941	9,051	10,053
Banbury Center	1,848	1,856	1,666	1,763	1,729
Dolan DNA Learning Center	1,392	1,471	1,878	2,978	2,564
Watson School of Biological Sciences	218	682	927	1,496	1,769
Total revenue	57,670	66,374	75,790	79,065	86,257
Expenses:					
Main Lab:					
Research and training	27,179	30,345	34,716	37,872	41,749
Operation and maintenance of plant	5,765	6,589	7,027	8,661	8,702
General and administrative	3,844	6,162	6,492	6,395	7,507
Other	7,863	7,075	9,505	8,550	8,959
CSHL Press	6,077	8,186	9,515	8,962	10,234
Banbury Center	1,614	1,702	1,536	1,597	1,616
Dolan DNA Learning Center	1,280	1,362	1,801	2,780	2,257
Watson School of Biological Sciences	218	682	927	1,496	1,769
Total expenses, excluding depreciation and amortization	53,840	62,103	71,519	76,313	82,793
Excess before depreciation, amortization, and designation of funds	3,830	4,271	4,271	2,752	3,464
Depreciation and amortization	(3,526)	(3,974)	(4,620)	(5,165)	(5,459)
(Designation) release of funds[1]	–	(297)	349	1,848	–
Net operating excess (deficit)	$ 304	–	–	(565)	(1,995)

The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor Laboratory prepares operating budgets.
[1]Funds designated to underwrite future direct and indirect expenses of new research programs.



COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
DECEMBER 31, 2003

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the Dolan DNA Learning Center receive a substantial portion of their funding through grants from the Federal Government and through grants, capital gifts, and annual contributions from private foundations, corporations, and individuals. The following section summarizes funding that occurred during 2003.

GRANTS January 1, 2003–December 31, 2003

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2003 Funding**
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Equipment	Dr. Stillman	09/01/03 08/31/04	491,782 *
Program Projects	Dr. Herr	01/01/77 12/31/06	4,895,303
	Dr. Stillman	08/01/87 07/31/05	3,742,281
Research Support	Dr. Cline	12/06/95 11/30/04	493,903
	Dr. Cline	03/01/98 03/31/06	530,316
	Dr. Enikolopov	08/01/99 05/31/04	434,547
	Dr. Enikolopov	09/12/94 11/30/04	409,907
	Dr. Enikolopov	03/11/02 12/31/03	207,500
	Dr. Grewal	03/01/00 08/31/03	64,821
	Dr. Hannon	09/01/00 08/31/05	307,600
	Dr. Hannon	03/01/01 02/28/03	166,000
	Dr. Helfman	09/01/99 06/30/04	409,631
	Dr. Hernandez	07/01/99 06/30/04	220,427
	Dr. Herr	03/01/02 02/28/06	298,800
	Dr. Hirano	07/01/01 06/30/05	296,348
	Dr. Hirano	05/01/96 04/30/04	417,585
	Dr. Huang	08/01/01 06/30/06	415,000
	Dr. Joshua-Tor	05/01/01 03/31/06	373,500
	Dr. Joshua-Tor	02/15/02 01/31/07	378,080
	Dr. Joshua-Tor	08/01/96 11/30/04	245,473
	Dr. Krainer	07/01/03 06/30/07	517,746 *
	Dr. Krainer	06/01/01 05/31/06	408,400
	Dr. Lazebnik	09/01/03 08/31/05	207,500 *
	Dr. Lowe	07/01/99 06/30/04	344,863
	Drs. Mainen/Brody	09/23/02 08/31/07	381,005
	Dr. Malinow	05/01/92 04/30/05	560,109
	Dr. Malinow	04/01/03 02/29/08	535,157 *
	Dr. Martienssen	08/01/03 07/31/07	337,166 *
	Drs. McCombie/Wigler/Zhang	01/01/99 12/31/03	471,428
	Dr. Muthuswamy	03/01/03 02/28/07	374,171
	Dr. Neuwald	09/30/98 08/31/06	373,500
	Dr. Skowronski	04/01/03 03/31/03	622,495 *
	Dr. Spector	04/01/03 03/31/07	605,785 *
	Dr. Stein	09/01/02 06/30/05	778,415
	Dr. Stein	09/24/02 09/25/03	130,253
	Dr. Stillman	07/01/91 05/31/04	552,156

*New grants awarded in 2003.

	Dr. Svoboda	12/01/98	11/30/03	338,325
	Dr. Svoboda	12/01/02	11/30/07	385,034
	Dr. Svoboda	06/01/03	03/31/08	315,351 *
	Dr. Tansey	05/01/03	04/30/07	328,250 *
	Dr. Tonks	08/01/91	03/31/06	627,701
	Dr. Tonks	05/01/97	06/30/05	348,600
	Dr. Tully	03/01/03	02/29/08	420,416 *
	Dr. Tully	08/01/03	05/31/07	379,125 *
	Drs. Tully/Zhang	09/28/00	06/30/03	283,149
	Dr. Van Aelst	05/01/03	04/30/08	337,088 *
	Dr. Wigler	05/01/03	04/30/07	485,815 *
	Dr. Wigler	09/30/02	07/31/04	166,000
	Dr. Wigler	05/01/99	07/31/03	996,358
	Dr. Xu	01/01/03	08/31/03	44,379 *
	Dr. Xu	08/01/01	07/31/05	317,320
	Dr. Xu	08/01/03	03/31/07	258,759 *
	Dr. Yin	09/30/02	07/31/04	166,000
	Dr. Yin	04/08/03	03/31/08	420,625 *
	Dr. Zador	01/23/03	12/31/07	420,000 *
	Dr. Zhang	08/01/00	07/31/04	403,616
	Dr. Zhang	09/30/97	08/31/03	768,529 *
	Dr. Zhong	02/01/96	05/31/04	292,073
	Dr. Zhong	07/01/03	06/30/08	345,000 *
Fellowships	E. Govek	05/01/01	04/30/03	24,766
	Dr. Kuhlman	12/01/02	11/30/05	46,420
	Dr. Lucito	06/01/02	05/31/07	146,534
	Dr. Ronemus	12/01/01	11/30/04	51,904
	Dr. Song	09/16/03	09/15/06	41,608 *
Training Support	Dr. Krainer	09/01/84	12/31/03	344,135
Course Support	X-ray Methods in Structural Biology	09/01/00	08/31/05	55,902
	Neurobiology Short-term Training Courses	07/01/01	06/30/06	160,528
	Cell and Developmental Biology of *Xenopus*	04/01/93	03/31/04	19,484
	Cancer Center Research Workshops	01/01/83	03/31/05	386,275
	Acquiring and Analyzing Genomic Sequence Data	04/01/95	03/31/04	93,069
	Immunocytochemistry, In Situ Hybridization	07/01/98	08/31/05	83,128
	C. elegans	08/01/98	07/31/06	73,382
	Bioinformatics: Writing Software for Genome Research	07/01/00	06/30/05	75,156
	Making and Using DNA Microarrays	09/30/99	06/30/04	55,551
	Computational Genomics	06/06/91	08/31/07	46,567
	Cellular Biology of Addiction	05/15/03	05/14/04	44,000 *
	Microarrays	09/30/99	06/30/04	55,551
	Proteomics	07/01/03	06/30/06	65,150 *
Meeting Support	Neurobiology of *Drosophila*	08/01/01	07/31/06	28,352
	Protein Phosphorylation and Cell Signaling	04/01/01	03/31/06	7,839
	68th Symposium: The Genome of *Homo sapiens*	05/15/01	04/30/06	5,000
	68th Symposium: The Genome of *Homo sapiens*	06/20/03	05/31/04	41,715 *
	The Ubiquitin Family	04/01/01	03/31/06	9,000
	Eukaryotic DNA Replication	08/01/01	07/31/06	10,000
	Mechanism of Eukaryotic Transcription	08/03/01	07/31/06	8,000
	Programmed Cell Death	08/01/01	07/31/06	12,000
	Telomeres and Telomerase	03/15/03	02/29/04	22,050 *

*New grants awarded in 2003.

	Learning and Memory	04/05/03	03/31/08	5,850 *
	Genome Informatics	04/01/03	03/31/04	20,850 *
	Eukaryotic mRNA Processing	08/01/03	07/31/08	9,000 *
	Rat Genomics and Models	09/30/03	09/29/04	19,990 *
NATIONAL SCIENCE FOUNDATION				
Research Support	Dr. Cline	09/01/99	08/31/04	117,137
	Dr. Helfman	02/01/99	01/31/04	118,662
	Dr. Jackson	08/01/02	07/31/05	116,659
	Drs. Martienssen/McCombie/Stein/Lucito	09/01/01	08/31/05	645,365
	Dr. McCombie	09/01/03	08/31/06	1,958,794 *
	Dr. Stein	09/01/02	08/31/04	360,148
	Dr. Stein	09/01/03	08/31/06	541,381 *
	Dr. Stein	12/15/03	11/30/07	676,203 *
	Dr. Stein	01/15/03	12/31/03	88,010 *
	Dr. Timmermans	07/01/02	06/30/05	119,967
	Dr. Timmermans	09/01/03	08/31/06	116,121 *
	Dr. Ware	09/01/03	08/31/08	339,522 *
	Dr. Zhang	09/15/03	08/31/08	195,000 *
Training Support	Undergraduate Research Program	01/01/03	12/31/03	50,000 *
	Dr. Sumazhim	09/01/03	08/31/05	50,000 *
	Dr. Vaughn	07/01/03	06/30/05	50,000 *
Course Support	Advanced Bacterial Genetics	05/01/99	04/30/04	90,494
	Arabidopsis Molecular Genetics	07/15/03	06/30/04	69,895 *
	Cell and Developmental Biology of *Xenopus*	09/01/02	08/31/06	22,159
Meeting Support	Genomics of Transcription Regulation	03/15/03	02/28/05	15,000 *
	Learning and Memory	04/01/03	03/31/04	11,206 *
	Telomeres and Telomerase	04/15/03	03/31/04	2,500 *
	Mechanisms of Eukaryotic Transcription	07/01/03	06/30/04	5,000 *
	Eukaryotic mRNA Processing	07/01/03	06/30/04	5,000 *
	Eukaryotic DNA Replication	09/01/03	08/31/04	5,000 *
	Neurobiology of *Drosophila*	09/15/03	08/31/04	15,987 *
UNITED STATES DEPARTMENT OF AGRICULTURE				
Research Support	Dr. Jackson	09/01/01	08/31/03	98,250
	Dr. Jackson	09/01/03	08/31/06	73,210 *
	Drs. Martienssen/McCombie	09/01/01	09/30/03	250,000
	Dr. Martienssen	12/01/00	11/30/03	85,000
	Dr. Martienssen	08/01/03	07/31/05	93,598 *
	Dr. Stein	09/01/00	08/31/04	838,016
	Dr. Stein	09/15/00	09/14/04	783,785
	Dr. Stein	10/01/02	09/30/04	220,000
	Dr. Stein	09/22/03	09/21/05	290,000 *
	Dr. Timmermans	09/01/01	08/31/03	65,960
UNITED STATES DEPARTMENT OF THE ARMY				
Research Support	Dr. Conklin	09/30/02	09/29/03	121,554 *
	Dr. Hamaguchi	04/15/00	05/15/04	61,165
	Dr. Hannon	06/01/00	07/01/04	121,554
	Dr. Hannon	04/01/02	03/31/06	721,742
	Dr. Hannon	06/01/00	05/31/04	71,134

*New grants awarded in 2003.

	Drs. Hannon/Lowe	09/01/02	08/31/05	166,598	
	Dr. Lazebnik	06/01/01	05/31/04	203,699	
	Dr. Van Aelst	09/01/01	08/31/03	182,662	
	Dr. Zhong	04/01/02	03/31/04	166,000	
Fellowship Support	M. Carmell	05/01/02	04/30/05	22,000	
	Dr. Du	06/01/02	05/31/05	50,000	
	Dr. Kannanganattu	01/01/01	12/31/04	49,796	
	E. Kim	04/01/03	03/31/06	28,500	*
	A. Lucs	04/01/03	03/31/06	29,162	*
	M. Moore	08/16/03	08/15/06	29,162	*
	Z. Nahle	07/01/02	06/30/05	22,000	
	Dr. Narita	07/01/01	06/30/04	51,601	
	P. Paddison	07/01/03	06/30/06	28,528	*
	Y. Seger	07/01/01	06/30/04	22,000	
	K. Siddiqui	04/01/03	03/31/06	29,162	*
	Dr. Silva	11/01/01	10/31/03	49,000	
	Dr. Xiang	06/01/03	05/31/06	55,372	*

MISCELLANEOUS GRANTS

Research Support

American Cancer Society	Dr. Wigler	01/01/96	12/31/03	10,000	
American Cancer Society	Dr. Wigler	01/01/01	12/31/05	70,000	
AKC Canine Health Foundation	Dr. McCombie	04/01/03	03/31/05	33,085	*
ALS Association	Dr. Enikolopov	04/01/03	03/31/04	50,000	*
Anonymous	Dr. Malinow	12/01/03	11/30/04	150,000	*
Breast Cancer Research Foundation	Dr. Wigler	10/01/03	09/30/04	250,000	*
Gladys Brooks Foundation	Library Support	01/01/03	12/31/03	83,393	*
The Charles A. Dana Foundation, Inc.	Dr. Cline	10/01/03	03/31/05	65,994	*
	Dr. Van Aelst	04/01/03	08/31/04	66,227	*
The Dart Foundation	Dr. Tully	05/01/03	04/30/06	2,233,333	*
DARPA/NYU Consortium Agreement	Drs. Wigler/Mittal	09/18/01	09/17/03	226,127	
Find a Cure Today (FACT)	Dr. Muthuswamy	07/01/03	06/30/04	22,400	*
Fraxa Research Foundation	Dr. Yin	08/01/03	02/29/04	25,000	*
Irving Hansen Foundation	Dr. Tansey	08/01/02	07/31/04	20,000	*
Jo-Ellen and Ira Hazan	Dr. Enikolopov	12/01/03	11/30/04	200,000	*
Helicon	Dr. Tully	07/01/02	06/30/03	168,000	
Human Frontier of Science Program Organization (HFSPO)	Dr. Mitra	6/15/03	06/14/05	96,473	*
	Dr. Spector	05/01/03	04/30/06	112,500	*
Immuno-RX	Dr. Mittal	08/01/02	07/31/04	57,869	
Juventis	Dr. Enikolopov	01/01/03	05/13/04	210,000	*
L.I.A.B.C. (Long Islanders Against Breast Cancer)	Dr. Wigler	01/01/03	12/31/03	127,437.33	*
The Leukemia & Lymphoma Society	Dr. Lowe	10/01/03	09/30/08	1,000,000	*
Long Beach Breast Cancer Coalition	Dr. Muthuswamy	09/01/03	08/31/04	1,000	*
The Lustgarten Foundation	Dr. Lucito	01/01/03	12/31/03	250,000	*
The Michael Scott Barish Human Cancer Grant sponsored by 1 in 9: The Long Island Breast Cancer Action Coalition	Dr. Wigler	01/01/03	12/31/03	16,002	*
The G. Harold & Leila Y. Mathers Charitable Foundation	Dr. Zador	07/01/03	06/30/06	163,144	*
The March of Dimes	Dr. Mills	06/01/03	05/31/06	68,686	*
McFarland Breast Cancer	Dr. Wigler	10/01/03	09/30/04	22,080	*
Merck & Co., Inc	Dr. Hannon	04/16/03	04/15/05	1,500,000	*
Met Life Foundation	Dr. Malinow	11/01/03	10/31/04	200,000	*
McKnight Endowment	Dr. Svoboda	08/01/02	07/31/04	100,000	*
Miracle Foundation	Dr. Wigler	07/16/03	07/15/04	100,000	*
Louis Morin Charitable Trust	Drs. Joshua-Tor/Dubnau	01/01/00	11/30/03	120,000	

*New grants awarded in 2003.

NAAR	Dr. Hatchwell	07/01/03	06/30/04	60,000	*
NIH/Baylor College of Medicine	Dr. Mills	09/01/01	08/31/06	214,356	
Consortium Agreement NIH/CalTech	Dr. Stein	07/01/03	06/30/08	548,831	*
Consortium Agreement	Dr. Stein	07/01/02	06/30/04	283,548	
	Dr. Stein	07/01/02	06/30/04	186,841	
	Dr. Svoboda	04/04/03	02/29/08	149,661	*
NIH/Columbia University Consortium Agreement	Dr. Lowe	09/30/00	07/31/05	502,210	
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Van Aelst	07/10/03	06/30/08	317,965	*
NIH/Northwestern University Consortium Agreement	Dr. Zhang	09/10/03	06/30/06	134,058	*
NIH/SUNY-Downstate Medical School Consortium Agreement	Dr. Yin	09/01/01	06/30/06	90,000	
NIH/Stony Brook University Consortium Agreement	Dr. Jackson	09/01/02	08/31/04	229,100	
NIH/University of Virginia Consortium Agreement	Dr. Stein	08/01/02	07/31/05	228,615	
NIH/University of California-San Diego Consortium Agreement	Dr. Zhang	09/01/01	08/31/04	130,315	
NIH/Washington University Consortium Agreement	Dr. Stein	03/01/99	10/31/03	45,348	
NIH/Washington University Consortium Agreement	Dr. Stein	09/30/99	10/31/03	17,058	
NIH/Washington University Consortium Agreement	Dr. Stein	11/01/03	10/31/06	38,109	*
Mr. and Mrs. Edmond J. Nouri	Dr. Tully	01/01/03	12/31/03	40,193	*
NSF/DOE/USDA/University of Arizona Consortium Agreement	Drs. McCombie/Martienssen/Stein	09/15/02	09/14/03	380,407	
NSF/University of Arizona Consortium Agreement	Dr. Stein	10/01/03	09/30/07	200,456	*
NSF/University of California-Berkeley Consortium Agreement	Drs. Jackson/Martienssen	10/01/01	09/30/06	275,785	
NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	09/15/03	08/31/04	20,000	*
NSF/University of Georgia Consortium Agreement	Dr. Timmermans	09/01/03	08/31/07	152,578	*
NSF/Rutgers University Consortium Agreement	Drs. Spector/Martienssen/ McCombie	10/01/00	09/30/05	418,392	
NSF/Stony Brook University Consortium Agreement	Dr. Jackson	09/01/02	08/31/04	229,099	*
NSF/University of Wisconsin Consortium Agreement	Dr. Martienssen	09/01/00	08/31/05	245,808	*
New York State Funds	Library Support	07/01/02	06/30/03	4,409	*
NYSTAR/Mt. Sinai School of Medicine Consortium Agreement	Dr. Svoboda	10/01/02	06/30/04	175,000	
NY Botanic Garden Plant Consortium	Dr. McCombie	03/01/02	12/31/03	9,335	*
OSI Pharmaceuticals	Dr. Hannon	10/21/03	10/20/05	450,000	*
Manyu Ogale	Dr. Wigler	07/01/03	06/30/04	250,000	*
The David & Lucille Packard Foundation	Drs. Chklovskii/Mainen/Zador	07/01/01	06/30/04	366,581	
The Perkin Fund	Women's Partnership for Science	06/01/02	05/31/04	37,500	
Pfizer, Inc.	Dr. Hannon	12/01/03	11/30/05	312,000	*
Rockefeller University Agreement	Dr. Mills	09/01/03	08/31/04	77,930	*
SAIC-NCI	Dr. Hannon	10/21/02	04/20/04	1,000,000	
The SNP Consortium, Ltd.	Dr. Stein	02/24/03	02/23/05	389,981	*
Seraph Foundation	Dr. Enikolopov	10/01/03	09/30/04	48,000	*
	Dr. Lazebnik	10/01/03	09/30/04	35,000	*
The Simons Foundation	Dr. Wigler	11/01/03	10/31/05	1,200,000	*
St. Giles Foundation	Dr. Hatchwell	03/01/03	02/29/04	150,000	*
Sungene Plant Consortium	Dr. Martienssen	07/01/00	06/30/05	135,000	

*New grants awarded in 2003.

Tularik, Inc.	Dr. Wigler	11/01/97	10/31/03	660,000
Tularik, Inc.	Dr. Wigler	01/01/02	12/31/03	500,000
USDA/University of Arizona Consortium Agreement	Dr. McCombie	09/15/03	09/14/04	223,758 *
The V Foundation	Dr. Muthuswamy	05/01/02	04/30/04	50,000
Whitehall Foundation, Inc.	Dr. Huang	08/16/01	08/15/04	75,000
Zeneca Plant Consortium	Dr. Martienssen	07/20/99	07/19/04	135,000

Fellowships

AACH Amgen Fellowship	Dr. Wendel	07/01/02	06/30/03	30,000
Alzheimer's Association	Dr. Boehm	10/01/03	09/30/05	50,000 *
	Dr. Ijima	10/01/03	09/30/05	50,000 *
Burghart Turner Fellowship	M. Juarez	06/01/03	08/31/03	3,000 *
Burroughs Wellcome	Dr. Yasuda	01/01/03	12/31/07	80,000 *
	Dr. Zito	09/01/02	07/31/04	58,000
CAP Cure Prostate Cancer	Dr. Lucito	01/01/02	12/31/03	100,000
CSHL Association Funds	Labwide Support	04/01/03	03/31/04	257,132 *
Cure Autism Now	Dr. Takahashi	06/01/02	05/31/04	49,201
Damon Runyon Cancer Research Foundation	Dr. Zuo	04/01/03	03/31/06	41,000 *
Demerec/Kaufmann/Hollaender Fellowship	Dr. Timmermans	01/01/03	12/31/03	13,310 *
Epilepsy Foundation	Dr. Haas	07/01/02	06/30/03	40,000
Fraxa Research Foundation	Dr. Bestman	09/01/03	08/31/04	35,000 *
Goldring Fellowship	Dr. Stillman	09/01/00	08/31/04	60,000 *
Helen Hay Whitney Foundation	Dr. Hemann	09/01/03	08/31/06	42,500 *
	Dr. Karpova	07/01/02	06/30/03	44,500
	Dr. Sheu	04/01/01	03/31/04	45,000
	Dr. Zito	07/01/00	06/30/03	45,000
Howard Hughes Medical Institute	Graduate Student Support	09/01/94	08/31/04	27,060 *
Human Frontier Science Program Organization (HFSPO)	Dr. Ango	04/01/02	03/31/05	42,000
	Dr. Becamel	04/01/03	03/31/06	42,000 *
	Dr. Cuvier	06/20/01	05/31/03	41,000
	Dr. Iijima	04/26/02	04/25/05	42,000
Jane Coffin Childs	Dr. Gillespie	07/01/02	06/30/03	42,500
Johns Hopkins/ Ellison Foundation Consortium Agreement	Dr. Kass-Eisler	01/01/02	03/31/03	126,359
Laurie Strauss	Dr. Hemann	04/01/03	03/31/04	15,000 *
Leukemia Research Foundation	Dr. Dickins	06/01/03	05/31/05	30,000 *
The Leukemia & Lymphoma Society	Dr. Buckley	11/01/03	10/31/06	40,000 *
	Dr. Duelli	12/31/00	12/30/03	35,000
	Dr. Losada	07/01/01	06/30/04	50,000
	Dr. Noma	07/01/02	06/30/03	17,750
	Dr. Scott	07/01/03	06/30/06	50,000 *
	Dr. Speck	07/01/01	06/30/04	40,000
	Dr. Tansey	07/01/01	06/30/06	100,000
Maxfield Foundation	Dr. Lazebnik	12/01/00	11/30/04	5,000 *
NARSAD	Dr. Barria-Roman	07/01/02	06/30/04	30,000
	Dr. Haas	07/01/02	06/30/04	39,110
	Dr. Piccini	07/01/03	06/30/05	30,000 *
NYSTAR	Dr. Henry	01/01/03	12/31/04	71,747 *
Pew Charitable Trust	Dr. Huang	07/01/02	06/30/06	60,053
Rita Allen Foundation	Dr. Hannon	10/01/00	09/30/03	50,000
Searle Scholars Program	Dr. Mainen	07/01/01	06/30/04	80,000
Seligson Special Fellow Award	Dr. Scott	03/17/03	03/16/04	75,000 *
Women's Partnership for Science	Dr. Timmermans	01/01/03	12/31/03	14,921 *
Wellcome Trust	Dr. Akerman	10/01/01	09/30/03	29,513
Wellcome Trust	Dr. Newey	10/01/01	09/30/03	26,306

*New grants awarded in 2003.

Course Support

Eppley Foundation	Magnetoencephalography Workshop	07/01/03	06/30/04	33,650 *
Howard Hughes Medical Institute	Neurobiology Courses	01/01/03	12/31/09	330,000 *
Esther A. & Joseph Klingenstein Fund, Inc.	Neurobiology Courses	03/01/02	02/28/05	50,000

Meeting Support

Abbott Laboratories	Microbial Pathogenesis Conference	2003	500 *
Abgent	Programmed Cell Death	2003	2,000 *
Amersham Biosciences Corp.	Structural Biology Discussion Group	2003	1,000 *
Biogen	Double Helix Conference	2003	25,000 *
Boston Biochem, Inc.	Ubiquitin and Ubiquitin-like Molecules	2003	1,500 *
Bruker AXA Inc.	Structural Biology Discussion Group	2003	1,000 *
Burroughs Wellcome	Microbial Pathogenesis Conference	2003	5,000 *
Cell Genesys, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	2003	1,500 *
Cell Signaling Technology, Inc.	Protein Phosphorylation and Cell Signaling	2003	2,000 *
	Programmed Cell Death	2003	1,000 *
Directgene, Inc.	Therapeutic Gene Delivery	2003	1,500 *
Discovery Partners International, Inc.	Structural Biology Discussion Group	2003	500 *
Genzyme	Therapeutic Gene Delivery	2003	1,500 *
Hampton Research Corp	Structural Biology Discussion Group	2003	1,000 *
Idun Pharmaceuticals, Inc.	Programmed Cell Death	2003	1,000 *
Institute for Biological Recognition and Catalysis, Inc.	Structural Biology Discussion Group	2003	1,500 *
Lexigen Pharmaceuticals Corp	Therapeutic Gene Delivery	2003	1,500 *
Lynn Caporale	68th Symposium: The Genome of *Homo sapiens*	2003	2,000 *
MSC Molecular Structure Corp	Structural Biology Discussion Group	2003	750 *
NSF/NYU Consortium Agreement	Genomics of Transcriptional Regulation	2003	16,889 *
Onyx Pharmaceuticals	Vector Targeting Strategies for Therapeutic Gene Delivery	2003	1,500 *
Rigaku/MSC, Inc.	Structural Biology Discussion Group	2003	750 *
Tecan U.S. Inc.	Structural Biology Discussion Group	2003	1,000 *
Vectorlogics, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	2003	1,500 *
Vertex	Programmed Cell Death	2003	1,000 *
VirRX, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	2003	2,000 *

Training Support

Bliss Fund	Undergraduate Research Program	2003	5,000 *
Dorcas Cummings	Undergraduate Research Program	2003	8,449 *
Dr. Ira Herskowitz	Undergraduate Research Program	2003	5,000 *
Jephson Education Trust	Undergraduate Research Program	2003	10,000 *

DNA 50TH GALA & EXHIBIT

The Charles A. Dana Foundation, Inc.	DNA 50th Gala	2003	100,000 *
Camille & Henry Dreyfus Foundation	NY Public Library DNA Exhibit	2003	35,000 *
The Ellison Medical Foundation	NY Public Library DNA Exhibit	2003	25,000 *
Morgan & Finnegan, L.L.P.	NY Public Library DNA Exhibit	2003	3,000 *
The Rockefeller University	NY Public Library DNA Exhibit	2003	10,000 *
The Rockefeller Foundation	NY Public Library DNA Exhibit	2003	25,000 *
Alfred P. Sloan Foundation	DNA 50th Gala	2003	25,000 *

*New grants awarded in 2003.

BANBURY CENTER

Grantor	Program/Principal Investigator	Duration of Grant	2003 Funding*
FEDERAL SUPPORT			
Brookhaven National Laboratory	Scientific Opportunities in Macromolecular Crystallography at NSLS-II	2003	$ 5,256*
Centers for Disease Control and Prevention (CDC)	Toward Understanding the Cellular and Molecular Mechanisms of Medically Unexplained Fatigue	2003	22,000*
NIH	Quantitative Genetic Networks	2003	25,000*
NIH–National Human Genome Research Institute	Eugenics, Genes, and Human Behavior	2003	23,202
NIH–National Institute of Mental Health (through a grant to FRAXA Research Foundation)	Synaptic Function in Fragile-X	2003	32,848*
NONFEDERAL SUPPORT			
Meeting Support			
Affymetrix, Inc.	Taking Cancer Genomics to the Clinic	2003	37,280*
The ALS Association	Finding New Genes Linked to Amyotrophic Lateral Sclerosis: A Focus on Current Technologies and Their Potential Application	2003	17,809*
Burroughs Wellcome Fund	Toward a More Unified Understanding of Infectious Disease	2003	30,847*
CFIDS Association of America	Toward Understanding the Cellular and Molecular Mechanisms of Medically Unexplained Fatigue	2003	5,000*
National Hypertension Association	Molecular Differentiation of Benign and Malignant Pheochromocytomas and Neuroblastomas	2003	3,760*
Redwood Neuroscience Institute	Neural Representation and Processing of Temporal Patterns	2003	41,632*
Albert B. Sabin Vaccine Institute, Inc.	Feasible Solutions to Global Vaccine Shortages	2003	33,595*
Alfred P. Sloan Foundation	Taxonomy and DNA Taxonomy, DNA, and the Bar Code of Life	2003	37,500*
The Swartz Foundation	Neural Circuits: Principles of Design and Operation	2003	42,632*
Verto Institute, LLC	The Biology of Neuroendocrine Tumors	2003	28,798*

*New grants awarded in 2003.

DOLAN DNA LEARNING CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2003 Funding*
FEDERAL GRANTS			
National Institutes of Health ELSI Research Program	Creation of *a Digital Image Archive on the American Eugenics Movement*	3/98–3/04	$ 187,773
National Institutes of Health	Creation of *Inside Cancer*	1/01–12/04	267,472
National Science Foundation	*Developing and Disseminating New Laboratories on Plant Molecular Genetics and Genomics*	2/03–1/06	124,390
NONFEDERAL GRANTS			
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	9/99–8/03	118,666
Howard Hughes Medical Institute	DNA Interactive Education Program	1/02–6/04	546,825
Pfizer Foundation	*Leadership Institute in Human and Molecular Genetics*	2/01–2/04	80,382

The following schools each awarded a grant for the *Genetics as a Model for Whole Learning* Program:

Allen Christian School	$ 350	Levittown Union Free School District	525
Bay Shore Union Free School District	1,920	Locust Valley Central School District	13,130
Baldwin Union Free School District	1,200	Long Beach City School District	700
Bellmore Union Free School District	2,550	Lynbrook Union Free School District	1,030
Bellmore–Merrick Central School District	12,400	Merrick Union Free School District	960
Bethpage Union Free School District	1,800	New York State School for the Deaf	240
Bnos Bais Yaakov	850	North Bellmore Union Free School District	1,050
Commack Union Free School District	2,131	Northport-East Northport Union Free School District	360
Crestwood Country Day School	350	North Shore Hebrew Academy	425
Crotona Achievement Center	75	Old Westbury School of the Holy Child	2,115
DGK Parochial School	125	Plainedge Union Free School District	1,375
East Williston Union Free School District	2,040	Port Washington Union Free School District	700
Elwood Union Free School District	3,400	Queens District #29	41,025
Farmingdale Union Free School District	2,585	Rockville Center Union Free School District	7,020
Friends Academy	3,790	Sand Creek Middle School	1,492
Garden City Union Free School District	9,765	Sayville Union Free School District	480
Great Neck Union Free School District	8,875	South Huntington Union Free School District	425
Grover Cleveland Middle School	960	St. Aiden School	700
Half Hollow Hills Central School District	5,250	St. Anne School	350
Harborfields Central School District	9,960	St. Edward the Confessor School	2,650
Hebrew Academy of Nassau County	850	St. Joseph School	350
Hempstead Union Free School District	350	St. Mary School	590
Herricks Union Free School District	1,750	St. Nicholas of Tolentine School	350
Holy Family Regional School	350	St. Peter's Kids	175
Huntington Union Free School District	480	Syosset Central School District	25,775
Jericho Union Free School District	7,025	Three Village Central School District	1,475
Kings Park Central School District	1,245	United Nations School	700
Laurel Hill School	350	West Babylon Union Free School District	350
Lawrence Union Free School District	7,010	Yeshiva Darchei Torah	850
Lawrence Woodmere Academy	240		

The following schools each awarded a grant for *Curriculum Study:*

Bellmore–Merrick Central High School District	$ 1,500	Locust Valley Central School District	1,250
Bethpage Union Free School District	2,350	Long Beach City School District	1,100
Commack Union Free School District	1,250	Oceanside Union Free School District	1,100
East Meadow Union Free School District	1,100	Portledge School	1,250
Elwood Union Free School District	1,100	Ramaz School	2,350
Friends Academy	2,350	Roslyn Union Free School District	1,250
Half Hollow Hills Central School District	2,350	Sachem Central School District	1,250
Herricks Union Free School District	1,100	South Huntington Union Free School District	1,250
Lawrence Union Free School District	1,250	Syosset Central School District	1,100

INSTITUTIONAL ADVANCEMENT

I have written in past annual reports about the philanthropy of individuals, corporations, and foundations making such a difference at Cold Spring Harbor Laboratory by increasing the pace of breakthrough science. This past year provides further testimony to this statement. The innovation in our education and research programs was propelled by an outstanding increase in the generosity of our supporters.

The gifts reported here are making substantial impact, enabling some of the best minds in biomedical discovery to advance the work to control cancer and to understand psychiatric and other disorders of the brain and neurological system, as well as educating tomorrow's research leaders. At such an important time in biomedicine, the increases in philanthropy reported here are particularly appreciated. With basic research becoming more closely linked with clinical improvements, this is a time when your generosity will bring real returns for our families and future generations.

Rod Miller, Vice President for Institutional Advancement

Cold Spring Harbor Laboratory is a nonprofit research and educational institution, chartered by the State of New York. Less than half of the Laboratory's annual revenues are derived from Federal grants and contracts, and thus we rely heavily on support from the private sector: foundations, corporations, and individuals. Contributions from the private sector are tax-exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory is designated a "public charity" and, therefore, is enabled to receive funds resulting from the termination of "private foundations."

Foundations, corporations, and individuals can give to Cold Spring Harbor through a variety of methods:

Gifts of Money can be made directly to Cold Spring Harbor Laboratory.

Securities: Stock certificates may be reassigned directly or transferred through your broker. Appreciated securities should be given outright, which will avoid capital gains taxes on the appreciated value. Securities that have decreased in value should be sold, and the proceeds donated. In this way, a donor will receive a deduction for both the loss and the charitable contribution.

Life Insurance: You may designate the Laboratory as the beneficiary of an existing or new policy, or irrevocably assign ownership of the policy. There are estate tax benefits in either case. If ownership is assigned, there is an immediate tax deduction.

Pooled Income Funds: Combine gifts from a number of donors in a pool for attractive investment and tax purposes.

Appreciated Real Estate or Personal Property: Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Charitable Remainder Trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Bequests: Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified.

Conversion of Private Foundation to "Public" Status on Termination: This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a supporting organization of Cold Spring Harbor Laboratory.

Matching Gifts: Many employers will match gifts to Cold Spring Harbor Laboratory and/or the Watson School of Biological Sciences. Please check with your employer to augment your gift.

For additional information, please contact the Office of the Vice President for Institutional Advancement, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6858.

CAPITAL AND PROGRAM CONTRIBUTIONS

January 1, 2003–December 31, 2003

Contributions of $5,000 and above, exclusive of Annual Fund

In 2003, Cold Spring Harbor Laboratory received significant support in the form of capital, program, and gifts-in-kind contributions from individuals, foundations, and corporations.

Anonymous (2)
Arrow Electronics
Holly H. Bard Fund
Michael Scott Barish Human Cancer Grant sponsored by 1 in 9: The Long Island Breast Cancer Action Coalition
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Lauri Strauss Leukemia Foundation
Dr. Waclaw Szybalski
The V Foundation
Dr. and Mrs. James D. Watson
The Whitehall Foundation
Women in Science Luncheon

Total	**$29,699,250.29**

* New pledges for 2003.

WATSON SCHOOL OF BIOLOGICAL SCIENCES CAPITAL CAMPAIGN

January 1, 2003–December 31, 2003

Contributions and pledges, exclusive of Annual Fund

The Watson School of Biological Sciences at Cold Spring Harbor Laboratory was established in 1998 to provide graduate education in the biological sciences at the Ph.D. level. The mission of the doctoral program is for students to gain extensive knowledge in biology through a combined research and education experience, with strong guidance from the faculty, and to complete the program in approximately four years. This accelerated program is designed to increase the number of highly skilled biologists available to apply the wealth of data from the human genome and other biological discoveries.

In its fifth year, the Watson School matriculated nine additional students, bringing the total number to 35. In 2003, the Watson School received significant support from individuals, foundations, trusts, and corporations.

Purpose	Donor
Beckman Graduate Studentships	Arnold and Mabel Beckman Foundation
Core Course	Arnold and Mabel Beckman Foundation
Dean's Chair	Annenberg Foundation Lita Annenberg Hazen Foundation
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Total	**$2,407,450.00**

* New pledges for 2003.

Corporate Sponsor Program

The Corporate Sponsor Program continues to provide critical funding for the vigorous meetings program held at Cold Spring Harbor Laboratory, whether at Grace Auditorium on the main Laboratory campus or at the Banbury Center. Without the strong foundation provided by the Program, we could neither plan with confidence for the year's meetings nor introduce new and unusual topics.

The Corporate Benefactor level continues to be attractive. The introduction of this upper level was stimulated by the continuing mergers in the pharmaceutical world, which have created very large companies and, at the same time, removed companies that had been contributors to the Program. Pfizer Inc. was the first company to join at this level and six other of the most notable pharmaceutical and biotechnology companies have contributed as Corporate Benefactors. The Corporate Sponsor contributors are no less important and we are especially proud that many of our current members have been long-term contributors. In 2003, we welcomed a new member, Lexicon Genetics, Inc., which stepped up from the Affiliate level.

The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for 16 representatives of the Corporate Benefactors and eight representatives of the Corporate Sponsors at our meetings. In 2003, no fewer than 20 meetings took place in Grace Auditorium. Six and three scientists, respectively, from Benefactors and Sponsors may attend meetings at the Banbury Center, where attendance is otherwise only by invitation of the organizers. Member companies receive gratis copies of Cold Spring Harbor Laboratory Press publications, including the journals *Genes & Development, Learning & Memory, Protein Science, Genome Research*, and *RNA*. We acknowledge our Sponsors in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, and this is mailed to approximately 17,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory Web site on the Meetings Office and Banbury Center pages. Members for 2003 included:

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Total	**$859,000**

Dolan DNALC Corporate Advisory Board Annual Fund

The Corporate Advisory Board, established in 1992, serves the Dolan DNA Learning Center as a liaison to the corporate community and assists in securing unrestricted support. As a means of raising support and awareness, the Corporate Advisory Board conducts the annual Golf Tournament and the Annual Fund.

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Total	**$103,931.00**

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THANK YOU
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Cold Spring Harbor Laboratory volunteers
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Total	**$134,311.05**

Total Dolan DNALC Annual Fund $238,242.05

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Association President's Report

2003 was a banner year for the Association. We celebrated the 50th Anniversary of the discovery of the double helix structure of DNA with the strongest community outreach effort to date. Our membership is now almost 800 strong and growing.

Thanks to this gratifying community support, we have raised more than $1,000,000 in unrestricted donations to advance the Association's mission of helping young scientists. Since the Laboratory receives only 40% of its funding from the government, Association support remains critical for the financial health of the institution. This year, the Laboratory once again won the second highest rating in the nation from *The Non-Profit Times* and a four-star rating from *Charity Navigator* for prudent and responsible operational and fiscal management. As donors, we can be assured that our donation dollars are being wisely used.

The Association gained strong new leadership in 2003 with the election of Directors Timothy S. Broadbent, Jay C. Plourde, John C. Stevenson, Ph.D., and Lynn McGrath Tone. Retiring Directors David Deming, Francis Elder, Bob Gay, Allen Jebsen, Ann Seifert, and Marjorie von Stade have enriched the Laboratory with their hard work and genuine commitment to the our mission. Each has been an inspiring and enthusiastic ambassador. A very special thanks to David, retired President of the Association, for his dynamic and wise leadership. It has been a privilege for me to work with him.

One of the perks of serving as Director is learning much more about our scientists' research. Several times a year, we enjoy informal lectures from young investigators, usually those whose work is supported by the Annual Fund. During the past year, we heard from:

- Rachel von Roeschlaub, a cancer researcher and genetics teacher, who discussed her recent trip to the foothills of the Himalayas to teach Tibetan monks about genes and DNA. Rachel, also a fine artist, supports her teaching in India with her painting sales.

- Sandy Kuhlman, a neuroscientist, who discussed how small changes in vision can have long-lasting effects on brain function in the young.
- Mary Byrne, a plant biologist, who discussed plant variation in form, i.e., branching patterns and leaf shape, showing how in studies with *Arabidopsis* and maize, her laboratory identifies genes involved in developmental processes for eventual application to crop propagation.
- Mike Packer, a cell biologist, who discussed his work with nitric oxide on control of stem cell proliferation with therapeutic possibilities for chemotherapy and transplantation. Mike was joined by his wife Jeana, another fine artist, whose varied and impressive canvases were presented at a show at the Laboratory in November.

Events for the year began on Sunday, February 9, with the Annual Meeting of the Association, chaired by Directors Lori Garofalo and Michael O'Brien. Harvard anthropologist Dr. Richard Wrangham presented his intriguing theory on the more rapid development of *Homo sapiens* after the discovery of fire, when cooked food was digested in significantly less time than raw food. The meeting was followed by a lively cocktail reception.

April 5 was the high point of the Association calendar with *Jazz at the Lab*. Special thanks for an exceptionally beautiful and well-organized evening go to Chairwoman Kate Friedman. Now in its fifth year, *Jazz* netted more than $70,000 for the annual fund, with record attendance topping 400 guests. We were fortunate to feature for the second year the legendary Joe Bushkin on piano, with Bob Merrill's vocals and trumpet. Others in the ensemble included Joe Cohn, John Colianni, Wycliffe Gordon, Jay Leonhart, Eddie Locke, and Karolina Strassmayer who played to exuberant encore requests from a packed house. The evening's financial success was due primarily to the generosity of our Underwriters, listed below:

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Dorian and Norman Feckl
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Cathy and Douglas Soref
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Diane and Bernie Yatauro

Mary Lindsay and Jim Large at *Jazz at the Lab*.

Bruce Stillman, Ginger Lucas, and Sandra and Steve Lessing at *Jazz at the Lab.*

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Janine and Roland Tatzel
Dori and Peter Tilles
Lynn and Pat Tone

First graders from the Goose Hill Primary School enjoyed two days of in-depth exploration with scientists in Bush Hall at six booths representing varying research during the second annual Science Fair in early May. More than 100 children enjoyed peering through microscopes, touching mammalian brains, viewing colorful protein structures through 3-D glasses, comparing mutant to nonmutant plants, and more.

The 26th annual Dorcas Cummings Memorial Lecture was held on Sunday, June 1, at the culmination of the CSHL Annual Symposium. Our good friend, Francis Collins, Ph.D., Director of The National Human Genome Research Institute, gave an especially entertaining lecture entitled, "The Human Genome, Where Do We Go from Here?" The timely lecture was one of the highlights of our 50th

*In kind.

Goose Hill first graders at CSHLA Science Fair.

Anniversary Double Helix celebration. It was at the 1953 Symposium at which James D. Watson announced his discovery of the structure of DNA. Following the lecture, 27 local families opened their homes for dinner parties for friends and scientists from around the world who had attended the week-long Symposium. Once again we had co-chairs Lynn Gray and Cynthia Stebbins to thank for their energetic and time-consuming outreach in organizing the dinner parties. Special thanks go to all our gracious and generous hosts and co-hosts, listed below, who continue one of the Laboratory's warmest traditions:

Marion and Frank Ahmed
Sophie and Dill Ayres
Mary Lenore Blair
Amy and Gerald Brogan
Barbara and Jim Brundige
Leah and Alan Butler
Trudy and Tom Calabrese
Rita and John Cleary
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Joyce and John Phelan
Julie and Luis Rinaldini
Cathy and Doug Soref
Jane and Jim Spingarn
Cynthia and Jim Stebbins
Janet and John Stevenson
Ingrid and Niklaus Vetsch
Elise and Hugh Wallace
Terry and Robert Walton
Kathy and Scott Wells

Our Partnership Picnic this year was hosted by Lola and John Grace. John, Association Vice-President, and Lola, Treasurer of the Board of Trustees, opened their lovely Oyster Bay Cove home and gardens to scientists and their "Partners." Partners are community families who befriend our young scientists, inviting them on occasion to their homes, sharing theatre tickets, books, etc. The sunny autumn day and setting were both picture perfect, as children romped and played on the rolling lawn overlooking Long Island Sound and grownups savored a delicious lunch.

September ended with our Annual Cocktail Party for major donors, hosted this year by Director Lori Garofalo and her husband Stephen, at their sprawling Mill Neck estate, *La Reserve*. Helping was their adorable daughter, Olivia, who charmed all the guests. A wonderful collection of many of the Laboratory's best friends mingled while enjoying the exquisite view from the Garofalos' balcony. Sandy Kulman, a CSHLA Fellow, spoke briefly to the group about her research and her life at Cold Spring Harbor Laboratory, thanks to the Association's support, and Bruce Stillman provided an update on Laboratory research.

Trudy Calabrese, Edith Seligson, and John and Kate Friedman at the Major Donor Party.

Major Donor Party hosts Lori and Stephen Garofalo.

The New York Committee is focusing outreach increasingly on individual major donors. To that end, Director David Banker and his wife Pamela put together a luncheon in honor of Jim Watson at the Knickerbocker Club in October. The Bankers' thoughtful guest list included old friends of the Laboratory as well as a number of new people with philanthropic inclinations. They all enjoyed an update on the Lab from Bruce Stillman and Jim Watson's insights for future implications of biomedical research.

Larry Remmel was the catalyst in November for Jim Watson's lecture at the Collegiate School on the Upper West Side. The daytime lecture for the student body was extremely well received and followed by a lively question and answer session.

The year ended with a holiday celebration at The Mary D. Lindsay Child Care Center. Several Directors participated, including the best Santa Claus ever, John Stevenson. It was touching to see the happy faces of so many "Lab Family" children. One of the missions of the Association is to help support the Child Care Center through a scholarship program funded by donations to the Annual Fund.

Special kudos go to Cathy Soref, our fellow Director, for her brainchild DNA STUFF. She conceived this idea of opening an independent, solely owned retail venture, stocked with creative and interesting merchandise related to the double helix. Cathy and her family will turn over the profits from their company as a contribution to the CSHL Annual Fund. We are indebted to Cathy for her creativity and energy on behalf of the Lab. Click on www.dnastuff.com/.

On a personal note, I wish to thank everyone at the Laboratory, neighbors, and friends for supporting the Association. We, in this community, are fortunate to have the Laboratory in our midst. It is a very special institution of scientific research and education, and an invaluable local resource. I am grateful for the opportunity and privilege of being a Cold Spring Harbor Laboratory volunteer.

Trudy H. Calabrese
President

Members of the Cold Spring Harbor Association

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Angels, Benefactors, Patrons, Associates*	$ 868,773.79
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Endowment Income	$ 8,825.00
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The President's Council was formed ten years ago to bring together leaders from business, research, and biotechnology who share an interest in science and Cold Spring Harbor Laboratory's research. President's Council members contribute $25,000 or more annually to support the Laboratory's *Cold Spring Harbor Fellows*—exceptional young Ph.D.s and M.D.s who are making great strides toward independent and important research. The 2003 meeting took place on May 17 and 18 and explored the topic, *The Mind of the Bird*. Our presenting scientists were engaging speakers and leaders in their field: John W. Fitzpatrick, Ph.D., Sidney Gauthreaux, Ph.D., Timothy Birkhead, Ph.D., and Irene Pepperberg, Ph.D. The following were members of the 2003 President's Council:

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Through thoughtful estate planning, the Harbor Society is ensuring a better quality of life for our families and future generations. This distinguished group of men and women helps extend the dramatic advances in molecular biology at Cold Spring Harbor with estate planning vehicles that maximize gift or estate tax benefits. Members of the Harbor Society have made bequests in wills or planned gifts of real estate, marketable securities, retirement plan assets, and art or antiques for tax-wise philanthropy that advances biomedical discovery.

In 2003, the Laboratory was delighted to record with appreciation a substantial charitable remainder trust for the benefit of the Watson School of Biological Sciences from the Chairman of the Board of Trustees, William Miller, and his wife Irene. The Estate of William L. Matheson and the Estate of Elisabeth Sloan Livingston also provided further support for the Laboratory. In the fall, Estate and Tax Planning seminars were held at the Woodbury Cancer Genome Research Center with many attendees subsequently visiting specific research laboratories.

The Laboratory also appreciates the support of the Planned Giving Advisory Group whose members provide ongoing advice in estate and tax planning matters.

Undergraduate Research Program

Founded in 1959, the Undergraduate Research Program allows college students from around the world to participate in hands-on inquiry-based avenues of exploration. This educational approach increases students' understanding of science, nurtures their enthusiasm about science, exposes them to the excitement of scientific discovery, and stimulates their curiosities, while interesting them in pursuing research careers or other science careers. Twenty-five participants composed of 14 men and 11 women stemming from Canada, Ireland, Lithuania, The Netherlands, Poland, Slovenia, the United Kingdom, and the United States spent ten weeks during the summer at Cold Spring Harbor Laboratory conducting research under the guidance of staff scientists.

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Patricia Crawford
Christie De Rosalia
Jennifer Donovan
Sandra Neuwald
Barbara Purcell
Theresa Saia
Rashmi Sinha
Rita Wienclaw

Information Technology

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Elizabeth Cherian-Samuel
Gregg Evans
Salvatore Fabozzi
James Gergel
Vsevolod Ilyushchenko
Derek Johnson
Juan Carlos Leon
Michael Malave
Louis Malfi
Matthew Newhall
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Chun Pong Cheung
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Catherine Eberstark
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Jan Eisenman
Paula Smith
Peter Stehli
Jill Stone
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Margot Bennett
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Cara Brick
Clare Bunce
Kiryn Haslinger
Margaret Henderson
Helena Johnson
Teresa Kruger
Marisa Macari
Gail Sherman
Rhonda Veros
Claudia Zago

Meetings and Courses

David Stewart, Executive Director
Edward Campodonico
Michael Cannavaro
Joseph Carrieri
William Dickerson
Gerald McCloskey
Andrew Mendelsohn
Maureen Morrow
Andrea Newell
Valerie Pakaluk
James Parsons
Jonathan Parsons
Lauren Postyn
Mary Smith
Margaret Stellabotte
Andrea Stephenson
Jenna Williams
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Philip Lembo, Director
Margaret Brock
Carol Brower
Susan DeAngelo
Jeffrey DuPree
Bruce Fahlbusch
Christopher Oravitz
Krystyna Rzonca
Barbara Santos
Leonard Sposato
Wanda Stolen

Special Events

Charles Prizzi, Director
Joan Lui
Elizabeth Panagot

Technology Transfer

John Maroney, Director
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