ANNUAL REPORT 2005

COLD SPRING HARBOR LABORATORY

COLD SPRING HARBOR LABORATO

ANNUAL REPORT 2005

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Front cover: Looking across Cold Spring Harbor at Knight House (*left*) and the newly dedicated Cutting House (*right*). Both serve as graduate housing for students in the Watson School. (Photo by Miriam Chua.)

Back cover: Additional graduate housing located on Uplands Farm dedicated in 2006 in honor of Elisabeth Livingston, William Miller, and Wendy Vander Poel Russell. (Photo by Miriam Chua.)

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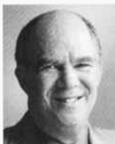
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*Deceased (2005)

H. Bentley Glass (1906–2005)

H. Bentley Glass, the distinguished geneticist and educator whose intimate involvement with Cold Spring Harbor Laboratory spanned more than 50 years, died on January 16, 2005, one day before he would have celebrated his 99th birthday. Born in Laichoufu, China to Baptist missionary parents, he first came to the United States as a student at Baylor University in Waco, Texas. After graduation, he taught science and coached football for 2 years at Timpson High School, near the Louisiana border, before returning to Baylor for research that led to his master's degree. Already focused on genetics, he moved in 1929 to the University of Texas at Austin for Ph.D. research under the supervision of already famed *Drosophila* geneticist Herman J. Muller, whose 1926 discovery that X rays induce mutations later led to a 1946 Nobel Prize. Because of his unorthodox social life and left-wing political affiliations, Muller's position as a professor at the University was increasingly unstable, leading to his 1932 move to the Berlin laboratory of Russian-born geneticist, N. Timofeev-Ressovsky. Bentley, whose later career was to mark him as the best of Muller's American students, used a highly prized National Research Council Postdoctoral Fellowship to also go to Berlin, then the scientific capital of the world. But just before he was to arrive, his potential mentor, the German-Jewish geneticist Curt Stern, fearful that the Nazis would come to power, abruptly decided to remain in the United States. Bentley briefly worked in Timofeev's lab until Hitler's rise to power in March 1933 led to his going on to Norway and the laboratory of the population geneticist Otto Mohr.

On returning to the United States, Bentley taught for 4 years at Stephens College in Columbia, Missouri where he married embryology graduate student Suzanne Smith. In 1938, they moved to Baltimore, Maryland and for the next 9 years Bentley taught at Gaucher College, a women's college located close to Johns Hopkins University. After World War II, he became a biology professor at Johns Hopkins, there organizing with Bill McElroy eight McCullum Pratt symposia on biochemistry, each of which led to influential books. Bentley also long presided over the *Quarterly Review of Biology* (from 1958 to 1986). His 13 years at Hopkins were his most productive as a scientist. Although he initially made his reputation as a *Drosophila* scientist, he later also left his mark as a human geneticist working on blood group polymorphisms, genetic drift, and the biological effects of ionizing radiation. Through this involvement in human genetics, Bentley became engaged in a number of public controversies concerning race relations, Lysenkoism, academic freedom, and nuclear fallout. In the late 1940s, he served as a highly active member of the Genetics Society of America's committee to counter antigenetic opinions, an action group created to stop the spread of Lysenko's influence beyond Soviet borders. At the same time, he was elected president of the Maryland Chapter of the American Civil Liberties Union and chaired the American Association of University Professors Special Committee on Academic Freedom and Tenure. He opposed loyalty oaths and refused an appointment to the Maryland Radiation Control Advisory Board because doing so would have required him to sign a loyalty oath.

In the late 1950s and 1960s, Bentley was deeply involved with major attempts to reform secondary school

science curricula. Early on, he chaired the Biological Sciences Curriculum Study, a collaborative project that produced three highly influential textbooks. Each volume focused not on memorization but on learning as a process. His other notable effort as an educator involved his membership in the Baltimore County School Board of Education from 1954 to 1958. In that capacity, he used his authority as a geneticist to argue for desegregation of the city's schools. Reportedly, he told the school board that until opportunity is equalized, no one can say what is inherent by natural law (genetic endowment). *The New Republic* was to describe him as a unique "triple threat" to defenders of the status quo in Maryland—by training, a scientist; by political affiliation, a Democrat; and by religious persuasion, a Baptist.

Bentley was the subject of several postwar FBI investigations, with his views on race relations rather than his outspoken support of academic freedom landing him on J. Edgar Hoover's security risk list. It was a $5.00 donation to the Communist-tainted American Youth for Democracy that kindled the FBI director's ire. Bentley paid that sum to attend a 1944 luncheon at which he spoke against segregation in the military. Although he was officially cleared in 1955, the FBI continued to monitor Bentley's activities throughout the 1960s.

Bentley's close association with Cold Spring Harbor Laboratory began in 1941, the first of four summers (also '42, '46, and '52) that he spent living in Williams House as a summer investigator. A participant in many symposia, he was the summarizer of the 1964 June Symposium on "Human Genetics." Long an informal advisor and confident of Milislav Demerec during the latter's 20-year directorship, Bentley was appointed in 1959 to an ad hoc review committee for advice on the future direction of the Lab. The committee, chaired by Edward Tatum of The Rockefeller University, deplored the lack of funds for senior staff positions and argued for continued emphasis on molecular biology and genetics.

Following his appointment as the academic vice president of Stony Brook University, Bentley was in a position to directly help the science of Cold Spring Harbor Laboratory. Soon joining our Board of Trustees, he succeeded Ed Tatum as chairman in October 1967—a critical time during which the Lab's existence into the future was widely doubted. John Cairns no longer wished to continue as director and no funds were available to pay the stipend of a successor. For the previous 2 years, I had unsuccessfully tried to find such salary monies. With the board now having a supportive chairman, I had the courage to propose that I become director as long as I could remain a full-salaried professor at Harvard. Bentley and Norton Zinder, who succeeded Tatum as Rockefeller's representative on our board, both instantly endorsed my plan for action. Franklin Ford, the Dean of Arts and Sciences at Harvard, happily gave his okay to proceed. Again, Bentley canvassed our board members about my taking the Lab's reigns, obtaining their unanimous consent.

During the remaining 5 years of Bentley's chairmanship, he and I, in contrast to John Cairns and Ed Tatum, never had significant disagreements. I particularly valued his support of a June 1973 meeting of our trustees. After sustained pressure by Town of Huntington Supervisor Jerome Ambro, the wealthy marina owner Arthur Knudsen agreed to sell us Whaler's Cove Yacht Club on the eastern edge of Cold Spring Harbor for $300,000. Were we not to buy it, Knudsen planned to double its size, bastardizing our harbor's still old-fashioned 19th-century atmosphere. John Cairns and I strongly felt that the Lab's long-term future demanded that the inner harbor remain the same. But our long-valued trustee, Walter Page, temporarily thought otherwise, citing the Lab's still-limited financial resources. If Walter's views were to hold sway over our trustees, I said, I would return full-time to Harvard. Wisely, Bentley did not allow the matter to come to a divided vote. Walter soon graciously dropped his objections, allowing me to breathe easier. By then, going back to Harvard was not a viable option for either me or the Lab.

After his retirement from Stony Brook at age 70, Bentley and Suzanne continued to live for almost 20 more years in their charming Setauket home on Long Island Sound. Bentley's retirement was never passive. For a decade, he commuted weekly from Stony Brook to Philadelphia to serve as director of the Genetics Archive Project of the American Philosophical Society. They were still nearby when we dedicated our Bentley Glass cabin in 1989. Six years later, following Suzanne's death, Bentley moved to Boulder, Colorado where his daughter, Louise Edgar, was living.

Bentley Glass's passion for intellectual excellence and honesty and for human fairness and decency should never be forgotten.

James D. Watson

Ernst Mayr (1904–2005)

Ernst Mayr, one of the 20th century's leading evolutionary biologists, died on February 3, 2005 at the age of 100. Born in Kempten, Germany on July 5, 1904, Mayr was early on fascinated by wildlife, particularly birds. He completed a Ph.D. in zoology from the University of Berlin in 1926 in only 16 months and, upon graduation, was offered the position of assistant curator of the Zoological Museum at the University of Berlin. The position offered Mayr the opportunity to travel to New Guinea to begin his work in evolution.

During two trips to New Guinea and the nearby Solomon Islands, Mayr braved much rough terrain and ghastly heat and humidity to classify and catalog the region's birds of paradise. There, he developed the concept of species still taught today: A group of individuals are capable of breeding with one another but not with others outside the group. He also pioneered the concept that new species develop when existing groups divide into populations so different that they are unable to interbreed, solving one of the mysteries to which Darwin alluded years before.

In 1932, Mayr came to the United States to take the position of associate curator of the Whitney-Rothschild Collection of the American Museum of Natural History in New York, eventually rising to curator. In 1953, he was appointed Alexander Agassiz Professor of Zoology at Harvard University, later serving 10 years as director of its famed Museum of Comparative Zoology. He retained his named chair until his death.

Mayr was one of the most prolific writers in biology. With more than 25 books and countless articles, he made his first big start through his masterful *Systematics and the Origin of Species* (1942), the logical successor to his close friend Theodosius Dobzhansky's seminal 1937 book *Genetics and the Origin of Species*. His writing career only went into full throttle upon his then-mandatory 1975 retirement. He was 76 when his high-powered *The Growth of Biological Thought* was published to great acclaim. Equally impressive was his *This Is Life*, which revealed that at 90 his writing skills had not diminished. On its jacket cover I endorsed it as an intellectually masterful overview of the big questions in biology written with clarity and verve. In his 95th year, *What Evolution Is* was published, written partially in Florida, where he increasingly went during winters following the death of his wife Gretel, to whom he was married for more than 60 years. His last book *What Makes Biology Unique* came out in his 100th year, when his legendary good health precipitately failed, depriving the world of the two more books whose outlines were already well-thought through.

He was recognized with many awards and accolades, including memberships in the National Academy of Sciences, the American Academy of Arts and Sciences, and the Royal Society of London. Throughout his career, he was awarded more than 16 honorary degrees, including the 2001 honorary Ph.D. bestowed upon him by his alma mater on the 75th anniversary of his original degree. His research earned the National Medal of Science, Gregor Mendel Medal, Darwin Medal, and Brewster Medal.

During his time in New York at the Museum of Natural History, Mayr and his family were regular summer visitors to Cold Spring Harbor Laboratory. He only missed one summer here between 1943 and 1952, one

of few researchers on site at the time who maintained active natural history research. In 1950, he joined the board of directors of the Lab's local governing board—the Long Island Biological Association—serving until 1958, and over a 15-year stretch he attended seven symposia. He is remembered today, not only for his use of Hooper House, but more importantly be-cause our main dining room in Blackford Hall has borne his name since 1995.

I first met Ernst, his wife Gretel, and their daughters Christa and Susie during the summer of 1948, my first at Cold Spring Harbor. My reading of *Systematics and the Origin of Species* during my senior year at the University of Chicago had made him my bird-watcher hero. I still then was a keen bird-watcher and initially disappointed to see Ernst showing little interest in local birds. Watching birds by then for him no longer meant hard science; it was how evolution occurred that made him jump.

The Mayrs were regular attendees of the Saturday night square dances below Davenport House, and they were daily seen on Bungtown Road on their way to swimming out to the Lab's raft just off Airslie, where Milislav Demerec and his family lived. When I came back to give my first public talk on the double helix at the June 1953 Symposium on "Viruses," Ernst was among the first acquaintances from my past to greet me in Blackford Hall. Excitedly, he told me about his impending move to Harvard, adding that I would be surprised by how much Christa, his eldest daughter, had changed during my 3 years in Europe. To say the least, she instantly caught my fancy when she and Susie arrived several days later with their mother. For the next 2 years, I spent increasingly more time at their home near Harvard or at their newly purchased New England farmhouse just across the New Hampshire state line. When Christa's junior year at Swarthmore led to her marriage to a German engineering student, I temporarily saw less of Ernst. But by then I was also at Harvard, with department meetings and lunches at the Faculty Club frequently bringing us together. Although many of our fellow biology department members did not initially welcome the intrusion of molecular biologists into their midst, Ernst was never so worried and took pride in Harvard becoming a world leader in molecular as well as evolutionary biology.

In a 2002 interview, Ernst reflected fondly on his time at the Labor-atory and the fascinating characters he met there—in particular, Barbara McClintock, Milislav Demerec, and Max Delbrück. He colorfully recalled the chronic summer housing shortage, fun on the Sandspit, and the memories of a devoted group of scientists who shared his time. "It was a marvelous atmosphere," he said, "because there were a number of places where people got together and talked." For those who knew him, though, part of the wonderful atmosphere was talking to Ernst himself.

He is already sorely missed for the breadth of his strongly felt convictions.

James D. Watson

PRESIDENT'S REPORT

From the eastern shore of Cold Spring Harbor and many vantage points on our beautiful campus, the emergence of a cluster of new buildings is now obvious. This exciting expansion of our research facilities flows naturally from the success of our current research and makes real a long-held dream.

A decade ago, the village of Laurel Hollow redefined our campus as a scientific and research zone distinct from its residential areas. Soon thereafter, the Board of Trustees initiated a strategic review of future use of the Laboratory's 113-acre main campus. This review determined that any future development would take place at the south end of the campus, close to existing buildings, to encourage scientific collaboration. By the fall of 2001, our Connecticut-based architectural firm, Centerbrook, had completed a vision for campus expansion that made efficient use of available space while retaining the "village of science" style that has come to define Cold Spring Harbor Laboratory. Now, because of the ingenuity of the Laboratory's recent science, our need for such expansion is very pressing.

The dramatic success of our Cancer Center offers opportunities to turn our attention to novel therapeutic and diagnostic approaches for controlling human cancer. In recent years, our research staff has begun collaborating with clinicians in many cancer centers worldwide. A number of new technologies developed at the Laboratory offer novel approaches to investigating diagnoses and treatments. One new technology is the RNAi library developed in Greg Hannon's laboratory, which makes it possible to turn off the expression of every human gene and thus identify those that, when inhibited, cause tumor regression or enhance the effects of chemotherapy. This approach will accelerate the rate of discovery of new molecular targets for drugs that fight cancer.

A new effort in human genetics by Michael Wigler's research group, in collaboration with Robert Lucito, uses newly developed, powerful techniques for identifying the number of copies of genes that exist in each individual. Classical Mendelian genetics have taught us that we all have two copies of most of our genes, but in 2004, Wigler, Jonathan Sebat, and colleagues discovered that this idea is too simplistic. In our individual genomes, we all have either missing genes or extra copies of genes, and the particular genes affected vary. This new aspect of human genetic variation, referred to as copy-number polymorphism (CNP), has important implications. One is that CNP may contribute to an individual's vulnerability to disease, an insight that has given fresh impetus to research on the genetics of autism, schizophrenia, and neurodegenerative Parkinson's disease. Another is that CNP may influence cancer cells. Cancer results from accumulated mutations in an individual's DNA and many of these mutations are copy-number variations. Using the new technology, such variations can now be quickly detected by scanning DNA taken directly from a tumor itself. This information will help physicians to identify the cellular origin of a tumor and decide on appropriate therapy, and help scientists to identify potential therapeutic targets.

Identifying potential targets is immensely valuable, but validating them as effective is still a major challenge. One approach has been to study hard-to-cure cancers in mice, cancers that attempt to mimic the genetics of human cancer cells and also reflect the clinical experience with human tumors. Laboratory scientist Scott Lowe has been a leader in this area, pioneering new work on leukemias, lymphomas, and liver carcinomas. Lowe has developed techniques for creating tumors in mice that contain combinations of genes that cause human cancers. This approach permits the discovery of new cancer-causing genes as well as the testing of novel therapeutic strategies. For example, Lowe and Hannon have collaborated with Scott Powers to block tumor growth using RNAi molecules directed against cancer-producing genes. Such mouse models are potentially powerful because anticancer drugs can be tested and validated in many combinations, something that is very difficult to achieve in human clinical studies.

The Laboratory's development of these technologies, coupled with our strong program in understanding the biology of cancer, has fashioned a new path for cancer therapy research. Technology development has also opened additional possibilities in neuroscience.

The current neuroscience program began at CSHL in the early 1990s. From an initial focus on the genetics of learning and memory in the fruit fly by Tim Tully and colleagues, it expanded to similar studies in rodents. Underlying learning and memory are processes that mold and strengthen

synaptic connections among neurons. These processes are being studied in several laboratories here at Cold Spring Harbor and the need to observe them at work in living animals led to the construction of the Marks Building to house a new program in innovative, high-resolution brain imaging. The success of this program created the need for computational studies of complex imaging data and for modelling the way in which neuronal networks in the brain respond to external stimuli. From this basis, our neuroscience research has grown to embrace investigations at a systems level into how the brain handles the immensely complex processes of normal cognition, such as decision-making and attention. This cutting-edge initiative, pioneered by Zach Mainen, Tony Zador, and Carlos Brody, will, I expect, eventually mesh with the new studies on the genetics of human cognitive dysfunction. Our 15-year-old neuroscience research program has been remarkably successful and now needs space to grow even further.

A third area of research that has prospered at Cold Spring Harbor and elsewhere over the last decade is bioinformatics. It is no longer possible to keep track of all the data and information published in biology. As a result, new science has emerged that employs practioners with backgrounds in biology, medicine, physics, computer science, and mathematics who use computers as their workbench. Scientists at Cold Spring Harbor, such as Lincoln Stein, have been instrumental in this revolutionary approach, developing, for example, methods to manage and analyze the huge amounts of data that have emerged from worldwide genome-sequencing. From their work on our campus have emerged databases revealing new knowledge of human genetic variation and of the evolutionary history of humanity. But these scientists are housed in converted apartments normally used to accommodate visitors to our campus; new facilities are required to maintain and expand this exciting and important area of research.

In addition to new research facilities, we also need permanent housing for our highly successful Watson School of Biological Sciences, as well as more on-campus accommodation and facilities for the approximately 8000 yearly visitors to our meetings and courses programs. We also believe there is an opportunity to establish a unique center for the history of molecular biology and biotechnology. Many of the important advances in molecular biology can be traced to the courses, meetings, and research that took place at Cold Spring Harbor from the late 1940s onward, and the Laboratory is thus a natural location for the archives of the founders of these fields. Mila Pollock, our energetic, enthusiastic librarian and archivist, has begun a number of new initiatives such as oral history, Web-based memory boards, and collections of archive materials that have enhanced our already rich collections. Short- and long-term development of these resources will not only benefit our graduate students, but also provide valuable reference material for scholars pondering the future of genetics and biotechnology.

The needs, opportunities, and initiatives outlined here have prompted a clever and sensitively constructed master plan (page 3) that shows how Cold Spring Harbor Laboratory will respond. Our vision, however, is tempered by the reality of the substantial financial resources required to achieve it, so the plan will be executed in phases, the first being the completion of research facilities close to the existing buildings.

Site preparation for the research buildings that is now so visible was approved about 1 year ago by the Village of Laurel Hollow, and during that process, local residents were informed via a series of meetings and mailings. Installation of the infrastructure for the entire site will be complete by summer 2006 and construction of six new research buildings (100,000 sq. ft.) will proceed shortly thereafter, with the aim of completion at the close of 2008 or early 2009. Extensive landscaping and some reforestation of the disrupted areas of the campus will begin in spring 2006.

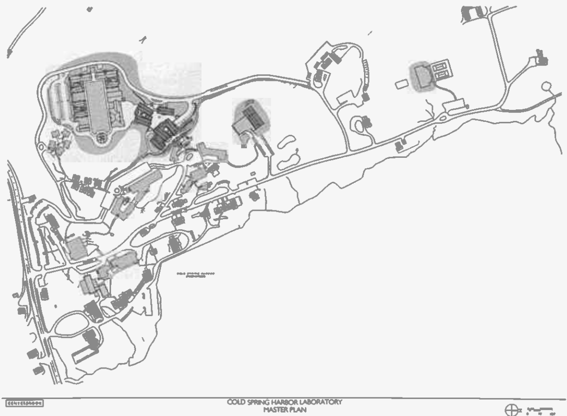
From the initial planning stages, through discussions with the Village and interactions with the architects, to the beginning of construction, the huge and complex needs of this unprecedented project have been addressed with extraordinary efforts from our administration and faculty and, in particular, the dedication, energy, and attention to detail of Art Brings, our Chief Facilities Officer. Without his hard work, this project would not be where it is today.

Throughout its history, Cold Spring Harbor Laboratory has expanded in response to significant advances in biological and biomedical research. This is such a moment. Our long-held dream is becoming reality and the opportunity to do even more exceptional research is energizing the faculty and support staff as never before. We have much to discover.

Existing conditions of the construction site

The Hillside Campus

The figure above shows the existing campus buildings. Route 25A and our southern boundary is on the left. The figure at right shows the new buildings in the master plan, shaded in light gray. Those planned as part of phase 1 are shown in dark gray: a series of six research buildings and a supporting mechanical building.



Plan of proposed modifications

HIGHLIGHTS OF THE YEAR

Research

Cancer

During the past few years, researchers have discovered that naturally occurring molecular snippets called "microRNAs" influence normal human growth and development. In 2005, CSHL scientists Greg Hannon, Scott Lowe, Scott Powers, and their colleagues discovered that microRNAs can also have an important role in cancer. It is a remarkable example of the progress that can be made when researchers working from different approaches collaborate closely on a project (in this case, Hannon, Lowe, and Powers working on microRNAs, animal models of cancer, and cancer genomics, respectively).

The study focused on a segment of human chromosome 13 that was known to be "amplified" or present in excess copies in several tumor types including B-cell lymphoma. The researchers observed that five microRNAs encoded by this DNA segment—referred to as the "mir-17-92 cluster"—are present at abnormally high levels in human B-cell lymphoma cell lines, as well as in biopsies of human lymphomas and colorectal carcinomas. These discoveries indicated that misregulated microRNAs might contribute to human cancer, particularly to B-cell lymphoma, but also to other forms of the disease. To test whether increased levels of the microRNAs could indeed contribute to cancer, the researchers engineered mouse cells to have high levels of the microRNAs. They found that the microRNAs accelerated tumor development and decreased survival in a mouse model of B-cell lymphoma. Moreover, lymphomas engineered to have high levels of the microRNAs consistently invaded organs including liver, lung, and kidney and lacked the extensive "programmed cell death" that otherwise keeps tumors in check. The observations suggest that microRNAs promote metastasis.

The Cancer Genome Atlas pilot project recently announced by the National Cancer Institute (NCI), in response to the recommendations of an NCI advisory committee of which I was a member, aims to find all of the major human cancer genes in specific types of cancers. Now, with the knowledge that genes encoding RNA can be among the set that promote cancer, the Cancer Genome Atlas has additional challenges to discover how widespread this phenomenon is.

In other work aimed at investigating cancer progression, Alea Mills and her colleagues have discovered that the loss of a gene called *p63* accelerates aging in mice. Similar versions of the gene are present in many organisms, including humans. The *p63* gene is thus likely to have a fundamental role in aging. Aging and cancer are two sides of the same coin. In one case, cells stop dividing. In the other, they cannot stop dividing. Therefore, Alea suspects that having the right amount of the p63 protein in the right cells at the right time normally creates a balance that enables organisms to live relatively cancer-free for a reasonably long time.

Alea Mills

To study how the *p63* gene works, the researchers devised a system for eliminating it from adult mouse tissues. What struck them right away was that the *p63*-deficient mice were aging prematurely. The effects of premature aging observed in these mice were hair loss, reduced fitness and body weight, progressive curvature of the spine, and shortened lifespan. The *p63* gene has been studied since it was discovered in 1997, but this is the first time it has been implicated in aging. A related protein called p53 is perhaps the most commonly mutated gene in human cancers and acts to suppress

tumor formation. Both p63 and p53 bind to specific sequences in DNA, and thus the interplay between them, as well as a third related protein p73, may set up a regulatory network that creates a balance between aging and cancer progression.

Genomics and Bioinformatics

Lincoln Stein

Bioinformatics researcher Lincoln Stein and his colleagues are part of a public-private effort—the International HapMap Consortium—whose aim is to chart the patterns of genetic variation that are common in the world's human population. In 2005, the researchers published the first comprehensive collection of their results called a "haplotype map" or HapMap. Comprising more than 1 million unique markers of human genetic variation, this first version of the HapMap is already accelerating the search for genes involved in common diseases including cancer, heart disease, diabetes, asthma, and macular degeneration. The HapMap may also be used to identify genetic factors that contribute to good health, such as those protecting against infectious diseases or other disorders.

In addition to its principal use as a resource for studies of human health and disease, the HapMap is yielding intriguing clues about how our species evolved over time and the evolutionary forces that were important as the human population spread around the globe. The HapMap is built on a foundation laid by the completion of the 3-billion-letter human genome sequence in 2003. As was the case with all of the data generated by the Human Genome Project, the data from the International HapMap Consortium are being made freely available to the world's scientists without restrictions.

The HapMap data are a collection of single-base changes, or SNPs, in the DNA that vary among individuals. These SNPs are inherited in blocks along chromosomes. They are a source of human genetic variation, but not the only source. Last year, Jonathan Sebat and Michael Wigler and their colleagues reported the discovery of a large number of deletions and amplifications of segments of the human genome. Called copy-number polymorphisms or CNPs, these also contribute to human genetic variation and to evolution of our species. Thus, we are now beginning to have a fairly robust view of how we as individuals differ from each other.

Infectious Disease

By determining the molecular structure of a protein that enables malaria parasites to invade human red blood cells, structural biologist Leemor Joshua-Tor and her student Niraj Tolia have uncovered valuable clues for rational antimalarial drug design and vaccine development. Niraj is a native of Kenya where malaria is endemic and a major medical problem, so he pursued this project with first-hand knowledge that something needs to be done about this devastating disease.

Niraj Tolia

Malaria causes approximately 400 million clinical cases and 2 million deaths annually, with more than 80% of deaths occurring among children. The disease is caused by mosquito-borne parasites of the genus *Plasmodium*. A major pathway through which malaria parasites invade red blood cells is the binding of a particular protein on the surface of parasites (EBA-175) to a receptor protein on the surface of red blood cells (glycophorin A). Consequently, drugs or vaccines that block the binding of EBA-175 to glycophorin A could be effective therapies for controlling malaria.

As a first step toward developing such therapies, Leemor's group used X-

ray crystallography to determine the atomic structure of a key portion of the EBA-175 protein. The results revealed precisely how the malaria parasite protein is likely to bind to human glycophorin A. This discovery enables researchers to take rational, structure-based approaches to designing antimalarial drugs and vaccines.

Cell Biology

David Spector and his colleagues have discovered a new molecular mechanism that is likely to control the production of many proteins in humans and other organisms. A deeper understanding of this rapid response, "cut and run" mechanism is predicted to have broad implications for biology and biomedical research. A few years ago, members of David's group noticed that under standard growth conditions, a particular subset of messenger RNA molecules lingered in the nucleus indefinitely (in structures called "speckles") and never reached the cytoplasm as they typically do. Spearheaded by the work of Kannanganattu Prasanth in David's group, a new paradigm of gene regulation was discovered that explains why such atypical messenger RNAs linger in the nucleus instead of being used immediately to produce protein.

First, they found that a mouse gene called *mCAT2* encodes two different kinds of messenger RNAs: a standard protein-coding version that is exported to the cytoplasm soon after it is produced in the nucleus and an atypical version that lingers in the nucleus. These observations led David and his colleagues to propose that when cells are stressed (e.g., by viral infection), perhaps the lingering *mCAT2* messenger RNAs are rapidly released from the nucleus and exported to the cytoplasm, thus circumventing the time-consuming process of producing new messenger RNA needed for stress response.

Confirming this idea, they discovered that the atypical *mCAT2* messenger RNAs in the nucleus were rapidly cleaved in response to interferon treatment (which mimics the effect of viral infection) and that the protein-coding portion of the messenger RNAs was indeed then rapidly exported to the cytoplasm and translated into protein. David has reason to believe that many genes in humans and other organisms that need to be rapidly induced are regulated in this way.

Because the protein-coding regions of most mammalian genes are interrupted by large segments of DNA (called introns), a phenomenon discovered at CSHL in 1977, leading to a Nobel Prize, it can take many hours to copy the DNA into mRNA. The new mechanism of gene regulation discovered by David and his colleagues may explain how genes can be rapidly expressed when cells receive an appropriate signal.

Plant Molecular Genetics

Rob Martienssen

In 1909, while harvesting a typical corn crop (*Zea mays*) in Illinois, a field worker noticed a plant so unusual that it was thought to be a new species. Its "peculiarly shaped ear" was "laid aside as a curiosity." The specimen was designated *Zea ramosa* (from the Latin *ramosus*, "having many branches"). Owing to the natural alteration of a single gene—later dubbed *ramosa1*—both the ear and the tassel of the *Zea ramosa* specimen were much more branched than those of typical corn plants, resulting in loose, crooked cobs and a far bushier than usual tassel.

Solving this enduring genetic mystery, Rob Martienssen and his colleagues recently isolated the *ramosa1* gene and discovered how it governs the architecture of corn plants. The discovery also explains why rice, other cereal crops, and even popular ornamental grasses look the way they do. The study was a

cover story of the international journal *Nature*. As part of the study revealed, to produce increasingly larger ears, primitive farmers almost certainly selected corn progenitors that bore special versions of the *ramosa1* gene: versions that restrained branching in the ear, thus enabling more rows of kernels to form.

Rob's group found that rice, corn, and other grasses have either none, some, or a comparatively large amount of *ramosa1* gene activity and that the levels and timing of this activity have a major impact on the architectural and agricultural yield of plants. It is a prime example of how selection by humans can influence the evolution of plant species. But importantly, the study also shows that considerable variation in plant structure can be caused by relatively small changes in the genomic DNA. Such simple variation in one or a few genes may also underlie the vast physical differences in domestic dogs that were also selected by man.

Neuroscience

According to a study by neuroscientist Partha Mitra and his colleagues, sleep helps young birds master the art of song, and it does so in a surprising way. The study revealed that when young zebra finches first awaken, instead of "picking up where they left off" in their vocal learning and memory, they are actually dramatically worse singers than they were at the end of the previous day. However, the quality of the birds' songs was found to improve after intense morning rehearsal such that by the end of each new day, their vocal performances are indeed better than the previous day.

More work is needed to explain this "one step back, two steps forward" effect of sleep on the brain circuits that govern vocal learning and memory consolidation. In the meantime, a useful analogy is the tempering of steel, in which to gain its ultimate structure and strength, it is first weakened. Vocal learning in songbirds is similar to speech acquisition in humans: Young birds go through a period of "screeching" before learning to imitate adult songs accurately, much as babies babble before grasping words. Therefore, to extend the significance of his discoveries to human development, Partha has begun to study the vocalizations of infant children in a rigorous, quantitative way. From this work, he expects to learn, among other things, how sleep affects learning and memory in humans.

Partha Mitra

To collect the data used in the zebra finch study, behavioral neuroscientists Ofer Tchernichovski and Sébastien Derégnaucourt of City College of New York recorded every sound—approximately 1 million syllables per bird—made by young zebra finches over several months as the birds learned to imitate and perfect their own renditions of recorded adult zebra finch songs. To measure how the vocal learning and memory of the birds changed over time, Partha developed mathematical algorithms that became the basis of powerful software he and Ofer created for analyzing the structure and patterns of recorded sounds (Sound Analysis Pro). Using this software, the vast amount of data was now accessible for interpretation, leading to their surprising discovery. Partha is a member of the Swartz Center for Computational Neuroscience, which supports research on development of theory and computational analysis of complex neuroscience data sets.

Another member of the Swartz Center, Mitya Chklovskii has made a significant advance in understanding the wiring of an entire nervous system of an animal. In the 1960s, Sydney Brenner started research at Cambridge University on the small worm *Caenorhabditis elegans*. This early beginning established the worm as one of the major model organisms for research on genetics, development, genomics, and neurobiology. For example, the entire lineage of cell divisions and cell deaths during development was determined by John Sulston

and colleagues, leading to the first description of how an animal grows from a fertilized egg and also led to the discovery of programmed cell death. Electron micrographs of the entire worm were collected as part of this project. Mitya and his colleagues used these to update and complete an analysis of the neurons and synaptic connections, thereby creating a wiring diagram of the worm's nervous system.

Previous research by Mitya and his colleagues had hypothesized that wiring of the nervous system was guided by placement of the neurons in the animal so that the wiring diagram minimized the length of the neuronal projections. This so-called wiring minimalization was proposed to be a strong evolutionary force in development of the worm brain, but also for neurons in our own cortex. Now, they have been able to test their model and, remarkably, find that their computer model fits the actual wiring of a majority of the worm's nervous system. Thus, computational predictions of how a nervous system is established can have a major role in understanding how neuronal networks come into being and how they function.

Cold Spring Harbor Laboratory Board of Trustees

As is customary, there were a number of new additions and departures to our Board of Trustees this year. We were pleased to appoint Dr. Robert Tjian, Howard Hughes Medical Institute Investigator and Professor in the Department of Molecular and Cellular Biology at the University of California, Berkeley, to our Scientific Trustees and Henry Wendt, III as our newest Honorary Trustee. Stephen M. Lessing, Managing Director, Lehman Brothers; David M. Rubenstein, Managing Director, The Carlyle Group; Alan Seligson, NAK International; and Andrew Solomon also began their terms as Individual Trustees this year.

Sadly, we said good-bye to Honorary Trustee H. Bentley Glass who passed away in January 2005 at the age of 98. Dr. Glass, one of the Nation's leading biologists, served as Chairman of the Board of Trustees in the 1960s, during a period of great expansion at CSHL, and actually convinced Jim Watson to join the Laboratory as its then new Director.

This year, Trudy Calabrese concluded her term as President of Cold Spring Harbor Laboratory Association (CSHLA), and therefore, her term as a member of the Board of Trustees. During her term, we saw the Annual Fund reach—and surpass—the $1 million mark, and we will always be grateful for her dedication and commitment to our organization. Stepping in to assume the role as President of the CSHLA, we welcomed Joseph T. Donohue, Managing Director of Gleacher Partners, LLC, to the Board of Trustees.

Hillside Campus Construction

2005 was a watershed year for construction projects at the Laboratory. After more than three years of planning and legal groundwork, led by Chief Facilities Officer Art Brings, the Laboratory's Hillside Campus Project began. Art's fantastic work, particularly with the Trustees of the Village of Laurel Hollow, has enabled the beginning stages of our construction to run smoothly. Construction progress for the entire project is on schedule, with a targeted completion date toward the end of 2008. 2005 saw the installation of much of the infrastructure for the entire project. This includes new roads, electricity, and water access and the construction of a series of drainage sumps that prevent water from directly running off our campus into the inner harbor of Cold Spring Harbor. This large drainage project reflects the long-time commitment by the Laboratory to protect the beautiful waterway that is our namesake.

Senators Dean Skelos, Kemp Hannon, and Carl Marcellino, and Charles A. Gargano, Chairman of Empire State Development, were in attendance at a groundbreaking ceremony

(*Left to right*) Henry Mund, Charles Gargano, James Watson, Bruce Stillman, Dean Skelos, Carl Marcellino, John Cleary, Dill Ayres, and Harry Anand at the groundbreaking ceremony

on July 14 to begin construction on The Center for Bioinformatics at CSHL, solidifying the efforts to boost the biotech industry on Long Island and celebrating the economic and employment opportunities created by this funding for all Long Islanders.

In preparation for our expansion, we have reconstructed our Development Office to prepare for the task of raising funds to build our new space. Chief Development Officer Charles V. Prizzi has done a remarkable job in directing our new efforts to raise funds, increasing our donor base and exploring new directions in fund-raising, and the department is now running very efficiently and most effectively.

Cancer Center Renewal

The NCI created a network of NCI-designated "Cancer Centers" to spearhead its efforts as the nation's leading agency that supports cancer research. In 1987, CSHL was approved to become an NCI-designated Cancer Center to allow expansion of the highly successful cancer research program initiated by Jim Watson when he came to Cold Spring Harbor in 1968. Since then, the CSHL Cancer Center has been one of the leading cancer research centers in the United States. The large NCI grant must be renewed every 5 years, and I am happy to report that the CSHL Cancer Center was reviewed again this year and approved for another 5 years of funding. The review characterized virtually all aspects of our Cancer Center as either "outstanding" or "excellent" and yielded the grant's highest ever priority score. For their efforts in this vital grant application process, I wish to acknowledge the enormous amount of effort from Scott Lowe (Professor and Deputy Director, CSHL Cancer Center), Denise Roberts (Deputy Director of Administration) and her assistant Denise Moller, together with Program Leaders Greg Hannon, Nick Tonks, and David Spector. I also thank Peter Sherwood (Director of Research Communications) who assisted with the writing of the introduction and the many Cancer Center members and staff who contributed to this successful grant renewal.

Awards and Honors

Greg Hannon

Scott Lowe

This year, Gregory Hannon and Scott Lowe were among the 43 biomedical scientists named Howard Hughes Medical Institute (HHMI) Investigators. The new Investigators were selected from more than 300 nominations drawn from 31 institutions nationwide, representing traditional biomedical research disciplines, as well as engineering, physics, chemistry, and computer science. In addition, both Greg and Scott were honored elsewhere this year. Scott received Memorial Sloan-Kettering Cancer Center's 2005 Paul Marks Prize for Cancer Research. This significant award recognizes researchers under the age of 45 for their major accomplishments in cancer research. Greg was named the 2005 recipient of the AACR Award for Outstanding Achievement in Cancer Research, which is given annually to recognize a young investigator on the basis of meritorious achievement in cancer research. *Esquire Magazine* also listed Greg as one of "America's Best & Brightest" in its year-end cover story.

Tatsuya Hirano and I were elected this year as Fellows of the American Association for the Advancement of Science.

CSHL neuroscientist and Director of Research Holly Cline was selected as a recipient of the 2005 NIH (National Institutes of Health) Director's Pioneer Award. Holly was among only 13 winners chosen from a pool of 840 scientists who underwent a rigorous selection process.

For the fifth consecutive year, the philanthropic evaluator *Charity Navigator* has bestowed their highest four-star rating on CSHL for its sound financial practices. This distinction ranks CSHL among the most responsible of more than 1.5 million philanthropic organizations that exist in America to date.

Barbara McClintock, the Nobel laureate who spent 50 years doing research at CSHL, was one of four scientists honored this year with a stamp from the United States Post Office. Dr. McClintock's work on transposable elements launched a new era of genetic discovery and this unique honor was well-deserved.

Dolan DNALC and Cold Spring Harbor High School Launch Partnership Program

This fall, Dolan DNALC Executive Director David Micklos initiated a new yearlong course in molecular genetics in collaboration with Cold Spring Harbor High School. The course is an advanced lecture and laboratory program open to high school seniors nominated by Cold Spring Harbor High School's science faculty. *Partnership* students learn the theory of molecular biology in the classroom and visit the Dolan DNALC every other day to put what they have learned into practice in the lab.

Development

Fund-raising this year set new heights, and we gratefully acknowledge the following for their major support of our campus expansion for research infrastructure and education: The Starr Foundation; The Frederick & Nancy DeMatteis Charitable Trust; Clay Mathematics Institute; Mrs. Leslie C. Quick, Jr. and family; Jeff Hawkins; Nancy Marks; Laurie J. Landeau; Eduardo Mestre; The William Stamps Farish Fund; William Randolph Hearst Foundation; Gladys and Roland Harriman Foundation; Francis Goelet Trust; Mr. and Mrs. Robert D. Lindsay; Mr. and Mrs. Donald Everett Axinn; Mary D. Lindsay; Mr. and Mrs. Gerard Leeds; The Koshland Foundation; Mr. and Mrs. John P. Cleary; and Edward A. Chernoff.

Private funding is also essential to maintain our vigorous research programs, enabling successful projects that are too innovative for public funding. For this reason, we are especially appreciative to those supporters who made major gifts in 2005 to our cancer and neuroscience research programs. We gratefully acknowledge donors of $100,000 or more to our cancer program: The Simons Foundation; The Karches Foundation for CLL Research; Fannie E. Rippel Foundation for Ovarian Cancer Research; The Frederick & Nancy DeMatteis Charitable Trust for Colon Cancer Research; Joan's Legacy: The Joan Scarangello Foundation to Conquer Lung Cancer; and The Miracle Foundation.

We also acknowledge the generous support to our neuroscience program including $100,000+ gifts from Mr. and Mrs. Theodore R. Stanley for schizophrenia research; The Swartz Foundation for neuroscience research; The Forrest C. Lattner Foundation for Schizophrenia Research; The Thomas Hartman Foundation; and Jo-Ellen and Ira Hazen.

President's Council

The President's Council brings together leaders from business, research, and biotechnology who share an interest in science and CSHL's research. Specifically, Council members support Cold Spring Harbor Laboratory Fellows—exceptional young and recently graduated Ph.D. or M.D. students who perform independent research at Cold Spring Harbor for a period of 3 years. This year, President's Council members raised an impressive $400,000 and established a new record for the annual contributions to this Fund. On page 426 we gratefully acknowledge all of the men and women who made this possible.

Robertson Research Fund

The Robertson Research Fund has been the primary in-house support for our scientists for more than three decades. During 2005, Robertson Funds supported research in the labs of Alea Mills, Cordula Schultz, Leemor Joshua-Tor, Rui-Ming Xu, and Wolfgang Lukowitz. In addition, the Robertson Fund supported the annual CSHL In-House Symposium and our programs for postdoctoral fellows and graduate students.

Sadly, we lost Carl Schafer this year to cancer. Carl was a long-time financial advisor to the Robertson family and Chairman of the Robertson Research Fund Committee here at CSHL. We all appreciated his positive and even-handed approach to various issues that arose over the years in connection with the management of the Robertson Research and Maintenance Funds. He represented the Robertson family well while, at the same time, making it clear that he also had the best interests of the Laboratory at heart. We are appreciative of all of his efforts on behalf of CSHL and his years of friendship.

Watson School of Biological Sciences

Support of the Dean's Chair, fellowships, and lectureships enable the Watson School to continue to grow and influence the field of biological sciences. We appreciate new gifts of $100,000 or more made this year by The Arnold and Mabel Beckman Foundation; Curt Englehorn; Bristol-Myers Squibb Foundation; the Lita Annenberg Hazen Foundation; and the Starr Foundation.

Dolan DNA Learning Center

Thanks to a very generous gift from the William and Flora Hewlett Foundation, progress can continue to be made on Dolan DNALC's newest addition to their Web portal: *Genes to*

Cognition (G2C) Online: A Network-driven Internet Site on Modern Brain Research. In addition, the Dolan DNALC received significant support from the Amgen Foundation for the Amgen Leadership Program.

Carnegie Library

In 2005, a grant from the Mellon Foundation provided consultants from two major library firms to assess our library systems and archival collection and to recommend software and computer systems to facilitate our digitization project. In addition, a 2-year $500,000 grant—*Preserving the Past and Present, Looking to the Future: Preserving and Digitizing the Cold Spring Harbor Laboratory Archives Collections*—from the Macy Foundation will allow us to create an integrated metadata database to provide worldwide online access to our digitized collection to scholars, students, and all other users. This year, Mila Pollock, our Librarian and Archivist, organized with Darwin Stapleton from The Rockefeller University and Jan Witkowski, Executive Director of our Banbury Center, a very successful international archives meeting. This meeting discussed approaches to maintaining archives in the modern electronic age and ways of funding the process. We also plan to establish a center for the history of Molecular Biology and Biotechnology at Cold Spring Harbor as part of our archives program, housing some of the most important archives at a place that has had a major role in these fields.

Additional Support

The Laboratory was fortunate to receive support for many ongoing projects in 2005 including a gift from Herbert J. Siegel for the Weghorn House; a gift from the Francis Goelet Trust for the Francis Goelet Fellowship in Biomathematics; a gift from the Gerber Foundation for congenital heart disease research; and a gift from the estate of Florence Strelkowski. We were also pleased to host a special concert given in Grace Auditorium by Manhattan School of Music students Victoria Sbarro and Evan Kory on October 16. "Playing for Parkinson's" benefited the Thomas Hartman Foundation for Parkinson's Research, which funds several of our neuroscience faculty as they investigate possible causes and cures for Parkinson's disease.

Breast Cancer Research Support

An essential aspect to our very successful breast cancer research program is the support we receive from local grassroots groups who provide direct research support for our program, in addition to the wonderful services they provide to the breast cancer community. This year, we were fortunate to receive support from the Breast Cancer Research Foundation; the Miracle Foundation; the Pierre and Pamela Omidyar Fund; 1 in 9: The Long Island Breast Cancer Action Coalition; Breast Cancer Help, Inc.; the Cold Spring Harbor Main Street Association; Dunkin Brands; Find A Cure Today (F.A.C.T.); The Islip Breast Cancer Coalition; The Long Island 2-Day Walk to Fight Breast Cancer, Inc.; Long Islanders Against Breast Cancer (L.I.A.B.C.); the Manhasset Women's Coalition Against Breast Cancer; Breast Cancer Awareness Day in memory of Elizabeth McFarland; the Waldbaum Foundation; the Judi Shesh Memorial Foundation; and the West Islip Breast Cancer Coalition.

Building Projects

In addition to the planned new construction, the Laboratory has completed several building projects during 2005. The student and postdoctoral fellow housing units at Uplands Farm

were completed after 2 years of construction and named in memory of long-time Laboratory friend and supporter Elisabeth (Betty) Livingston. In addition, two existing houses on Uplands Farm and another across the harbor from our main campus were named for other long-time supporters William and Irene Miller, Wendy Vander Poel Russell, and George Cutting. These residences enable our young scientists to live near the campus in affordable and attractive housing. The Laboratory also purchased a single-family residence adjoining its campus, renovated it, and rededicated it as the Garden House for use as faculty housing.

Other ongoing projects made significant progress during the year. At Banbury Center, the pool was reconstructed and the hostess quarters of the Robertson House were renovated. More than half of the extensive renovations planned for the James Laboratory have been completed, with the remainder scheduled to be completed in 2006. The renovation of the Demerec Tissue Culture Facility was completed. The bluestone patios at the front and rear of the Grace Auditorium were replaced, improving the spaces for our meetings visitors.

Special Events

Symposium

The 70th Symposium—"Molecular Approaches to Controlling Cancer"—once again included the annual Dorcas Cummings lecture. Charles Sawyers' outstanding lecture on "Making Progress through Molecular Attacks on Cancer" was presented to a mixed audience of scientists and lay friends and neighbors of the Laboratory. Following the lecture, more than 20 of our neighbors graciously opened their homes and hosted dinner parties for Symposium participants and Laboratory friends alike.

Gavin Borden Visiting Fellows

The 11th Annual Gavin Borden Visiting Fellow Lecture—in memory of the publisher of *Molecular Biology of the Cell*—was held on Wednesday, March 9. Dr. Huda Zoghbi, a Professor of Pediatrics, Neurology, Neuroscience, and Molecular and Human Genetics at the

Huda Zoghbi, Bruce Stillman, Lilian Clark Gann

Baylor College of Medicine, gave this year's lecture entitled "Cross-species Studies to Unfold the Pathogenesis of a Neurodegenerative Disease."

Public Lectures

The CSHL Cultural Series is a tradition in which an eclectic mix of artists, writers, and scientists present lectures, concerts, and exhibits that provide compelling glimpses of how we experience, discover, live in, and make sense of our world. Open to the public, the aim of the Cultural Series is to stimulate, inspire and entertain.

Rodney Brooks

April 19 Nina Federoff, Evan Pugh Professor at Penn State University: *Mendel in the Kitchen: Myths & Realities of Genetically Modified Food.*

May 16 Don Axinn, author of seven volumes of poetry and two novels: *Discussion & Screening of the Film* SPIN.

May 17 Rodney Brooks, director of the MIT Computer Science and Artificial Intelligence Laboratory and the Fujitsu Professor of Computer Science at MIT: *Flesh & Machines: How Robots Will Change Us.*

May 23 Sherwin Nuland, Clinical Professor of Surgery at the Yale University School of Medicine and Fellow of the university's Institution for Social and Policy Studies: *The Artist Looks at the Doctor: A Millennium of Clinical Observation.*

May 31 Cynthia Rosenzweig, Senior Research Scientist at the Columbia University Earth Institute and a professor of environmental sciences at Barnard College: *The Heat is On: Present & Future Impacts of Global Warming.*

Sept. 13 William Li, President, Medical Director and Cofounder, the Angiogenesis Foundation: *Canines & Cancer: New Therapies from & for Man's Best Friend.*

Sept. 20 Stephen S. Hall, contributing writer and editor at *The New York Times Magazine: Short People: Biological, Psychological, and Cultural Considerations.*

Sept. 27 Diana Reiss, Director of Marine Mammal Research and Conservation at the New York Aquarium of the Wildlife Conservation Society: *Exploring the Dolphin Mind.*

Oct. 11 Steve Squyres, Professor of Astronomy at Cornell University and the Principal Investigator on the Mars Exploration Rover Project: *Life on Mars: Lessons from the Rovers Spirit & Opportunity.*

Concerts

Joseph Lin and Benjamin Loeb

April 24	Gilles Vonsattel, piano
April 30	Benjamin Loeb and Joseph Lin, piano and violin
May 7	Jennifer Check and Ken Noda, soprano and piano
May 21	Jose Franch-Ballester, clarinet
August 27	Anton Barakhovsky and Sonya Ovrutsky, violin and piano
September 10	Timothy Fain, violin
September 17	Dmitri Berlinsky, Elena Baksht, and Suren Bagratuni, violin, piano and cello
September 24	Elizabeth Joy Roe and Greg Anderson, four-hands piano
October 8	Jean-Efflam Bavouzet, piano
October 29	Daxun Zhang, bass

Other Lectures

Jeff Hawkins

Laboratory Trustee Jeff Hawkins (creator of the Palm Pilot, the Treo smart phone, and other handheld devices) presented a fascinating lecture, *The World Is a Song: How Music Led to a Theory of Human Intelligence*, based on his latest book, *On Intelligence*. The lecture was held in February at The Juilliard School in New York City.

A panel discussion on *Memory—and How to Keep It!*—featuring Ronald Hedgepath, Ph.D., neuropsychologist at St. Johnland Nursing Center; Zach Mainen, Ph.D., Associate Professor at CSHL; Tracey J. Shors, Ph.D., Professor at Rutgers University; and Trey Sunderland, M.D., Director, Litwin-Zucker Alzheimer's Research Center, North Shore–LIJ Health System was held in Grace Auditorium on July 26.

A Memory Board advisory committee, which included Liz Watson and several community and library staff members, held an event entitled "Memories under the Moonlight," on July 28 to promote the Library and Archives' new Memory Board Web site. After a brief presentation by Tim Tully on the scientific aspects of memory, Mila Pollock described how to navigate the memory board site. The audience had the opportunity to relate their own humorous or poignant anecdotes of the Laboratory, evoking heartfelt emotions from both Laboratory and local community members, including Jim Watson and CSHL Honorary Trustee David Luke.

Craig Hinkley ("Gene Recipes: How Much Is a Pinch of RNA"), Partha Mitra ("How the Song Bird Sings"), Sandra Kulhman ("Rhythms in Nature and Our Body: Why Does Your Stomach Growl at Noon"), and Wolfgang Lukowitz ("Everything You Wanted to Know About Flowers [But Were Afraid to Ask]") all participated in our lecture series for fourth- to sixth-grade students and their parents, co-hosted with the Cold Spring Harbor School District, at the Dolan DNALC.

Rounding out our year, Dr. Kanta Subbarao, Senior Investigator in the Laboratory of Infectious Diseases at the National Institute of Allergy and Infectious Diseases, presented a timely and relevant public lecture, *Bird Flu and the Global Threat of Emerging Respiratory Diseases* on December 3.

Exhibits

The 2004 Photographer-in-Residence Reuben Cox exhibited his works in Bush Auditorium throughout the month of July. The large-scale photographs of many CSHL researchers were captured during his residency the previous summer.

Laboratory Employees

Chancellor News

CSHL Chancellor Jim Watson spent a great deal of time this year traveling to raise funds for CSHL research. In addition, Jim edited and wrote the foreword to *Darwin: The Indelible Stamp; The Evolution of an Idea*, a seminal volume on the famous biologist. Jim appeared on *The Charlie Rose Show* with Edward O. Wilson, the author of another recently released book about Darwin entitled *From So Simple a Beginning: The Four Great Books of Charles Darwin*, to discuss creationism, Darwinism, and science education. It was a riveting and very well-received program.

Jim Watson

New Staff

Sydney Gary, a neuroscientist who worked with Susan Hockfield at Yale, joined the Laboratory as Assistant Director of the Banbury Center. Sydney is working on developing the neuroscience and mental health meetings and courses programs, at Banbury and on the main campus.

Promotions

A number of faculty members were promoted in 2005, including Leemor Joshua-Tor, William Tansey, and Rui-Ming Xu, to Professor; Carlos Brody, Josh Huang, Alea Mills, and Marja Timmermans to Associate Professor; and Jonathan Sebat to Assistant Professor. Uwe Hilgert was also named Assistant Director of the Dolan DNALC.

Departures

As was announced last year, Professors Nouria Hernandez and Winship Herr moved to Switzerland to become professors at a new institute in Lausanne. During the year, Nouria was named director of the Center for Integrative Genomics. Assistant Professor Masaaki Hamaguchi left the Laboratory to assume a position at Fordham University.

This year saw the departure of Katya Davies, the long-term hostess at Robertson House at our Banbury Center. Katya was at the laboratory for 26 years and was known to many scientists who visited Banbury to attend meetings and courses. Katya created her own style of welcome for the numerous visitors that will long be remembered. We wish her well in retirement in Maine.

Community Outreach

CSHL sent a team to the fifth annual Pancreatic Cancer Walk at Old Westbury Gardens on July 31. The walk benefited The Lustgarten Foundation, a generous supporter of CSHL's research.

A group of volunteers manned a rest stop at the Long Island 2-Day Walk to Fight Breast Cancer, a 35-mile walk to benefit several groups across Long Island including CSHL. Our volunteers passed out water and raised spirits during the last legs of their journey.

Laboratory employees donated 489 pounds of assorted food through a food drive run by the Hauppauge Industrial Association to benefit Long Island Cares. We continued to support the Ronald McDonald House at Schneider Children's Hospital in New Hyde Park throughout the year by collecting empty printer, fax, and copy machine ink and toner cartridges, as well as old cell phones, for the foundation. Proceeds from the sale of these items went directly to the Ronald McDonald House at Schneider.

Long-term Service

The following employees celebrated milestone anniversaries in 2005:

35 years	Madeline Wisnewski
25 years	Bruce Fahlbusch, Michael Riggs, Linda Rodgers, Marlene Rubino, Andrea Stephenson
20 years	Frank Carberry, Lisa Manche, David Spector, Wanda Stolen, Harry Wozniak

(First row, left to right) Michael Riggs, Danuta Slodkowska, Karen McKeon, Barbara Purcell, Dora Merlino, Marlene Rubino, Lisa Manche, Linda Rodgers, Holly Cline. *(Second row, left to right)* David Spector, Harry Wozniak, James Watson, Madeline Wisnewski, Bruce Stillman, Ronald Romani, Frank Carberry, George Newell, Bruce Fahlbush

15 years June Blanchford, Clare Bunce, Edward Campodonico, Karen McKeon, Dora Merlino, George Newell, Natalia Peunova, Barbara Purcell, Ronald Romani, Danuta Slodkowska, Yew Yeo, Nicholas Tonks

Looking Forward

Our new research buildings at CSHL will be in the elegant style that has established the Laboratory as one of the most attractive research institutes in the world. Although the next few years of construction will be partially disruptive of our idyllic life of research and scholarship on the shores of Cold Spring Harbor, we look forward to the new facilities providing much needed research space for our outstanding research programs. I cannot wait until the new addition to our campus is completed and landscaped.

Bruce Stillman, Ph.D., F.R.S.
President

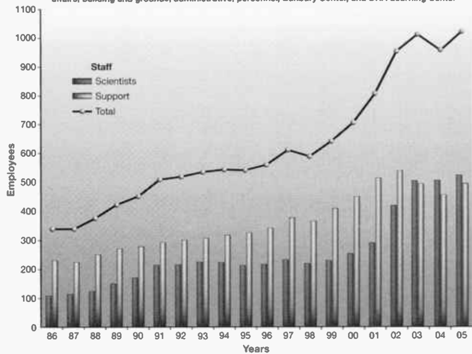
CHIEF OPERATING OFFICER'S REPORT

We are sure to remember 2005 as the year we broke ground on the largest expansion of the Laboratory's facilities in its 115-year history. This is an enormous undertaking. Five years of architectural and engineering planning, municipal and environmental reviews and approvals, and internal strategic and financial planning culminated in a July groundbreaking ceremony attended by New York State, Nassau County, and Village of Laurel Hollow leaders and officials.

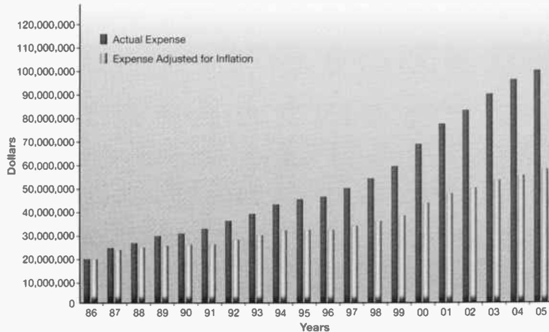
The process to date has been challenging but remarkably smooth. It has been facilitated by a diligent but cooperative Board of Trustees in the Village of Laurel Hollow. Centerbrook Architects have again put forth beautiful and innovative building designs. Chief Facilities Officer Art Brings and his staff have worked extremely effectively with the municipalities, government agencies, architects, engineers, and contractors to keep the project on time, on budget, and orderly. If all goes according to schedule, we will be dedicating and opening six new research buildings on the southern tip of our main campus in the fall of 2008. Approximately 100,000 square feet of new space will accommodate the expansion of our research programs in cancer, cognitive neuroscience, and bioinformatics. Fund-raising for the project is in full swing. Although the cost of infrastructure and buildings is approximately $100 million, we must devote substantial additional funds to start-up expenses and endowment. This is an ambitious undertaking, but we are encouraged by the generous gifts received to date. We are grateful for the support, energy, and generosity of the Laboratory's friends and Trustees who are so critical to the success of these efforts.

2005 also marked another successful year in our core programs. The research continues to be innovative and of the very highest quality. It has been recognized as such through distinguished faculty publications, awards, and honors too numerous to mention. Despite growing competition around the world, our Meetings and Courses program remains the most impactful and sought after in the field. The Banbury Center and the Dolan DNA Learning Center are operating at capacity as they each do their part to fulfill the Laboratory's educational mission. The Watson School of Biological Sciences has now established itself as one of the most innovative, popular, and successful graduate programs in biology. The Cold Spring Harbor Laboratory Press had a banner year, with its journals and books contributing very positively to our cash flow and bottom line.

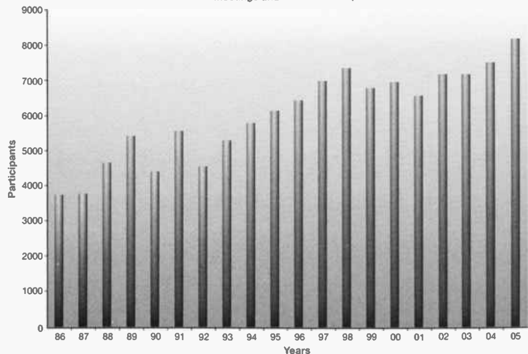
For the fiscal year ended 12/31/05, the Laboratory budget reached $103 million—a 7% increase over the prior year and well ahead of forecast. We had expected an operating deficit for the year, which did not materialize. This is a fortunate and interesting outcome given the fact that we were concerned up to the end of the year about the funding for the research program. As is often the case, fundings from grants and from endowment support were not going to be sufficient to cover the direct cost of the research in 2005. In such years, we must rely on royalty and licensing income to make up the difference. Fortunately, at year-end 2005, we received an unexpectedly large royalty payment on an expired patent that allowed us to cover the annual research budget in its entirety. We also anticipated a shortfall for the year in our ability to cover our indirect (or overhead) expenses. Despite rigorous and effective efforts to contain administrative expenses, it did not appear that there would be sufficient indirect



Operating Expense



Meetings and Course Participants



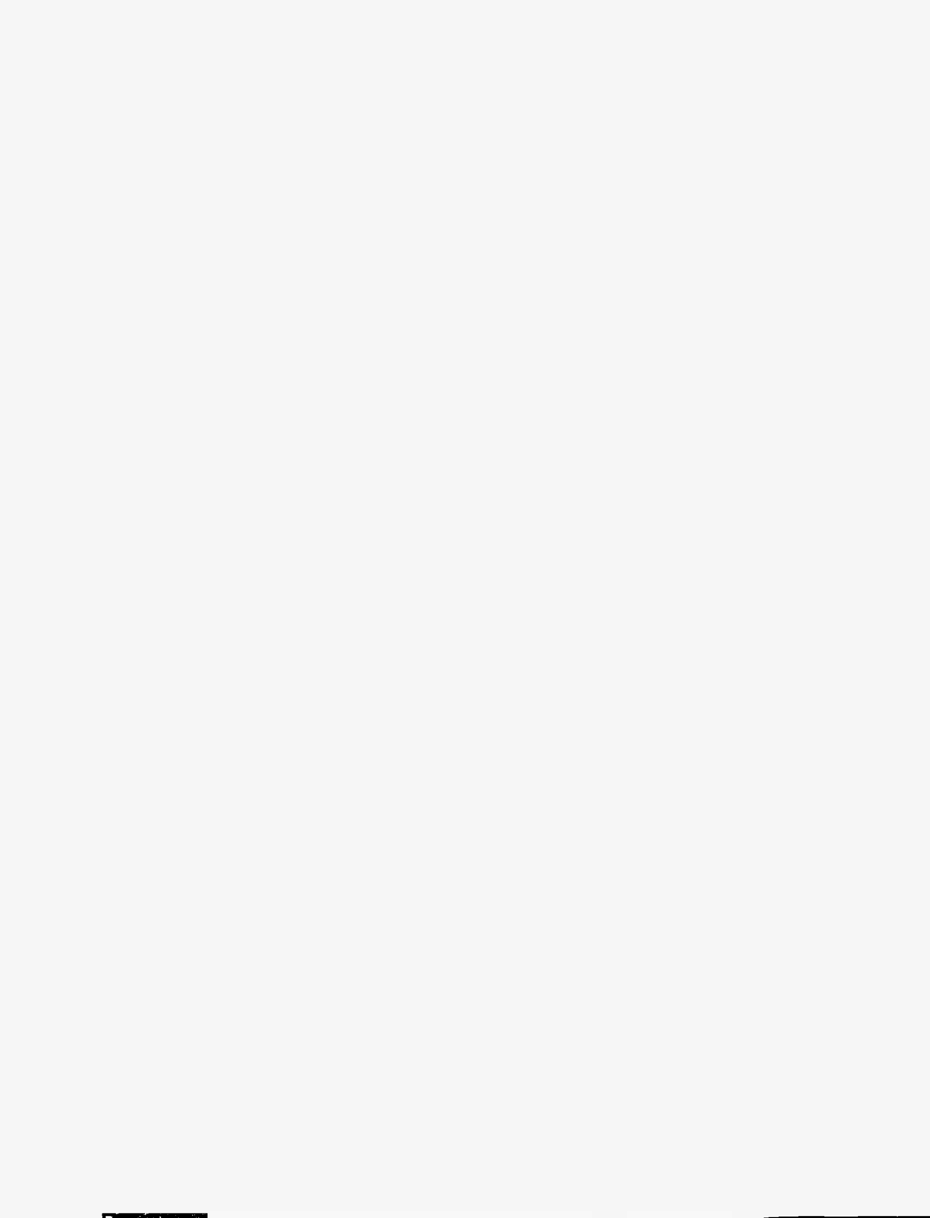
cost recovery on grants and income from operations to balance the budget after depreciation and interest expense. Fortunately, this also proved not to be the case. Indirect cost reimbursement was greater than budgeted, and the Cold Spring Harbor Laboratory Press had an excellent year, exceeding its budgeted operating surplus.

Investment returns on our endowment funds were strong for the third consecutive year. The total return on the portfolio was 7.5% for the calendar year—well ahead of the benchmark indices. Our total equity portfolio was up 9.1% as compared to the 4.9% appreciation in the S&P. This was a result of excellent performances from both our domestic and international equity managers. The fixed-income portfolio returned 3.1%, slightly ahead of the benchmarks. At year end, the market value of the endowment funds totaled $255 million after taking our customary annual draw of 4%. The Investment Committee continues to work diligently, under the leadership of Board Treasurer Ed Travaglianti, to establish a well-diversified portfolio of investments with proven managers and acceptable levels of risk and return. During the coming year, the Committee will be evaluating the allocation to alternative investments and deciding whether to increase the exposure to this asset class. Although investments in private equity, venture capital, real assets, and hedge funds can generate higher returns and less correlation, they are not always as accessible to endowments our size as they are to multibillion dollar funds.

Although it is satisfying to report a "balanced budget" for the year, our challenges going forward should not be underestimated. The cost of doing cutting-edge research is escalating at a time when the pool of federal funds for research is no longer growing. Operating costs that are difficult to control, such as utilities, insurance, and healthcare, are increasing at double-digit rates. To mount a successful fund-raising campaign, we have doubled the expense of our development office. And in 2006, we anticipate issuing bonds to support the building project that will more than double our interest expense. All of these hurdles present themselves at a time when we are undertaking a 40% expansion of our physical plant.

The good news is that Cold Spring Harbor Laboratory continues to distinguish itself by doing some of the world's best and most important scientific research. And in our experience, funding follows good research. In periods when public funding is static, we must increase our efforts to develop private sources of research support. Fortunately, we are already having success in this effort with names like Simons, Dart, Starr, Davis, Stanley, and Swartz funding important research initiatives. We must also continue to be creative in our efforts to commercialize our technology and intellectual property where possible. Our Office of Technology Transfer, so ably led over the years by John Maroney, has been very proactive and innovative in this area, allowing us to support research that would not otherwise have been possible. The future looks bright and exciting, but we have our work cut out for us.

Dill Ayres
Chief Operating Officer



RESEARCH

See previous page for photos of the following scientific staff:

Row 1: Hysall Oviedo; Md Aftabul Haque; Jody Barditch, Florinda Chaves; Jonathan Kui; Noah Gray, Takashi Sato

Row 2: Christian Speck; Gidon Felsen; Anna Kloc; Lidia Nascimento; Hongjae Sunwoo; Dominik Duelli; Michael Hueber

Row 3: Prakash Kumar; Min Yu; Alexis Maizel; Yue (Cindy) Wang; Tal Nawy; Anne Daulny; Joseph Simorowski

Row 4: Jeanne Wiggins; Z. Josh Huang, Caizhi Wu; Kandasamy Ravi; Darren Burgess; Inessa Hakker; Allison Blum; Benjamin Boettner

Row 5: Mary Kusenda; Rahul Sinha; Ingrid Ibarra; Rebecca Ewald, Kasandra Burgos; Taneisha James; Wen Xue, Mona Spector

Row 6: Victoria Aranda Calleja; Martin Bayer; Christopher Johns; Gilbert Henry; Alexandra Lucs; Byeong Ha Lee

Row 7: Theresa Auletta; Ross Dickins; Yi-Chun (Maria) Chen; Kathryn Bambino; Wei Wei

Row 8: Itay Onn; Oliver Fregoso; Khalid Siddiqui; Ingrid Ehrlich; Stephen Hearn; Leopoldo Petreanu

CANCER: GENE EXPRESSION

Sometimes complex processes are best studied by stripping them down to their essential components. Nouria Hernandez has done just that by biochemically reconstituting the process of RNA transcription with purified proteins in a test tube. These studies revealed the minimum requirements for transcription and uncovered two new components in the RNA polymerase III transcription pathway, the structural protein actin and the enzyme casein kinase II (CK2), which Hernandez has subsequently shown can both positively and negatively regulate transcription.

This year, Leemor Joshua-Tor determined, in collaboration with Greg Hannon, that an enzyme called Argonaute2 and a guide RNA are all that are needed to carry out the molecular slicing that cuts messenger RNA in an ultimate gene silencing step mediated by RNA interference. However, the researchers also found that the sliced messenger RNAs produced by Argonaute2 require another unknown protein to assist their release from the Argonaute2-guide RNA complex. In other work, Joshua-Tor's group revealed new clues to antimalarial drug design and vaccine development. These findings are based on the determination, by X-ray crystallography, of the molecular structure of a protein that enables malaria parasites to invade human red blood cells.

One of the ways cells adapt to various conditions is to produce different proteins from the same genetic message. This is accomplished by a mechanism called alternative splicing in which a single premessenger RNA molecule can be spliced in diverse patterns to produce messenger RNAs that encode different proteins. Adrian Krainer studies a family of splicing factors called SR proteins that bind to specific sites on messenger RNAs and recruit the splicing machinery to those sites. Recently, his group discovered that when present in excess, certain SR proteins can transform cells into a cancerous state. In collaboration with Scott Powers, Krainer's group is exploring the possibility that the splicing defects they have observed in human colon tumors identify novel therapeutic targets.

Rui-Ming Xu studies how proteins known to be involved in cancer are also integral parts of the protein complexes that maintain chromosome structure. By using X-ray crystallography, a tool for determining the three-dimensional structure of proteins and protein complexes, his group has determined the structures of a number of human oncoprotein complexes including Bmi-Ring 1B and EMSY-HP1 (the latter involved in hereditary breast cancer). These structural studies provide important insights into the functions of these proteins in normal cells and their roles in cancer.

A collaborative effort between Scott Lowe and William Tansey established how particular mutations in a potent growth control protein called Myc can lead to cancer. The series of Myc mutants the researchers studied came from patients who had developed Burkitt's lymphoma. Tansey became interested in the mutants because the corresponding genetic changes led to greatly increased levels of Myc protein in the cell, a phenomenon he had previously studied. When the scientists recapitulated the behavior of the human mutant Myc proteins in mice, they discovered that this class of *myc* mutations inactivates the "programmed cell death" pathway that normally acts to rid cancerous cells from the body.

Michael Myers is using the accumulated knowledge of how proteins and protein complexes regulate cell physiology to create global network maps of all the interacting proteins in a cell. These maps display how protein–protein interactions contribute to normal cell physiology, and how the interactions are disrupted in cancer. His global protein network map is beginning to reveal why cells can tolerate certain kinds of errors, whereas other defects lead to cancer. Myers aims to understand how such networks generate robustness (error tolerance) and adaptability in cells.

Jacek Skowronski studies how the AIDS virus exerts control over protein sorting and signaling machineries in human cells. He has recently identified several human proteins that associate with the AIDS viral proteins Nef and Vpr/Vpx. Skowronski's group is now working to verify these interactions and to determine their relevance to previously known roles of the Nef and Vpr/Vpx accessory factors in AIDS pathogenesis.

MECHANISMS OF TRANSCRIPTION

N. Hernandez F. Emran, B. Ma, S. Mayilvahanan, K. Samudre, A. Saxena, Y. Sun, C.-C. Yuan

RNA polymerase III (pol III) synthesizes a collection of essential RNA molecules that are themselves components of the protein synthetic and RNA processing machineries, such as the ribosomal 5S RNA genes, tRNA genes, and other genes including the U6 small nuclear RNA (snRNA) gene. Because most pol III transcripts are both abundant and stable, pol III transcription is highly regulated with cell growth and proliferation. In resting cells, only the small number of pol III transcripts that decay over time must be replaced, and pol III activity is low. In contrast, in rapidly dividing cells, an entire complement of the abundant 5S, tRNA, and other molecules must be synthesized in just one generation time, and pol III transcription is highly active. Indeed, pol III transcription is invariably up-regulated in malignant cells.

pol III can be recruited to three main classes of promoters. Classes I and II are present in the 5S RNA and tRNA genes, respectively, and are gene-internal. These promoters recruit a multisubunit complex known as TFIIIC, either directly in the case of the class II promoters or with the help of another transcription factor known as TFIIIA in the case of class I promoters. TFIIIC in turn recruits Brf1-TFIIIB, an activity containing the TATA-box-binding protein TBP, the TFIIB-related factor Brf1, and the SANT domain protein Bdp1. The resulting protein/DNA complex can then specifically recruit pol III. Class III promoters are present in the human U6 snRNA genes and other genes and are gene-external. They consist of two essential elements, a proximal sequence element (PSE) and a TATA box. Transcription from class III promoters can be reconstituted in vitro with recombinant snRNA-activating protein complex ($SNAP_c$), a PSE-binding factor composed of five types of subunits; recombinant Brf2-TFIIIB, an activity similar to Brf1-TFIIIB but in which Bfr1 is replaced with Brf2; and a highly purified pol III complex isolated from HeLa cells expressing a doubly tagged pol III subunit. When combined, these factors are sufficient to direct several rounds of correctly initiated and terminated transcription from the U6 promoter.

Class III promoters are remarkable in that they are highly similar to the promoters of many snRNA genes such as the human U1 and U2 snRNA genes, which are recognized by pol II. The U1 and U2 core promoters contain a PSE but they lack the TATA box that characterizes the pol III class III promoters. Transcription from the U1 and U2 promoters requires TBP, TFIIB, and many of the other factors required for pol II transcription from the classical pol II promoters, those that direct the transcription of mRNA-encoding genes. We are interested in understanding the mechanisms that govern the regulation of pol III transcription, as well as those that ensure recruitment of the correct RNA polymerase.

FACTORS IMPLICATED IN THE REGULATION OF snRNA GENE TRANSCRIPTION

In an attempt to identify regulators of snRNA gene transcription, we generated cell lines expressing tagged $SNAP_c$ subunits and used these cell lines to purify $SNAP_c$ and associated polypeptides by tag affinity chromatography. Our results indicate that $SNAP_c$ is associated with Yin-Yang-1 (YY1), a factor implicated in both activation and repression of transcription. Recombinant YY1 accelerates the binding of $SNAP_c$ to the PSE. Moreover, it enhances formation of a complex on the U6 snRNA promoter containing $SNAP_c$, TBP, Brf2, and Bdp1, as well as that of a subcomplex containing TBP, Brf2, and Bdp1. YY1 is found on both the pol II U1 and pol III U6 promoters as determined by chromatin immunoprecipitations. Thus, YY1 represents a new factor that participates in transcription complexes formed on both pol II and pol III promoters.

THE DETERMINATION OF RNA POLYMERASE SPECIFICITY

The transcription factors TFIIB, Brf1, and Brf2 share related amino-terminal zinc ribbon and core domains; however, as described above, TFIIB bridges pol II with

the promoter-bound preinitiation complex, whereas Brf1 and Brf2 are involved, as part of Brf1-TFIIIB and Brf2-TFIIIB, in the recruitment of pol III. Brf1 and Brf2 both have a carboxy-terminal extension absent in TFIIB, but their carboxy-terminal extensions are unrelated. In yeast Brf1, the carboxy-terminal extension interacts with the TBP/TATA box complex and contributes to the recruitment of Bdp1. The role of the carboxy-terminal extension of Brf2, if any, was unknown.

To understand how the U6 promoter can specifically recruit pol III, we tested truncated Brf2 as well as Brf2/TFIIB chimeric proteins for U6 transcription and for assembly of U6 preinitiation complexes. Our results characterized functions of various human Brf2 domains and revealed that the carboxy-terminal domain is required for efficient association of the protein with U6 promoter-bound TBP and $SNAP_c$ and for efficient recruitment of Bdp1. Close inspection of the carboxy-terminal domain of Brf2 revealed that the carboxy-terminal region of Brf2 contains a short region that can be aligned with conserved region II of Brf1, the Brf1 region required for efficient binding to the TBP/TATA box complex. The results suggest that a common feature among TFIIB family members involved in pol III transcription is the use of their carboxy-terminal extension to associate efficiently with a TBP/TATA box complex and to recruit Bdp1. This in turn suggests that the carboxy-terminal extensions in Brf1 and Brf2 are key to specific recruitment of pol III over pol II.

RNA polymerase specificity is, then, determined at two levels. The first level is the arrangement of the promoter elements themselves. In the human snRNA promoters, it is the presence or absence of a TATA box downstream from the PSE that largely determines pol III or pol II transcription, respectively. This in turn somehow results in the specific recruitment of either TFIIB in the case of pol II snRNA promoters or Brf2 in the case of pol III snRNA promoters. Ironically, we do not yet understand how the presence of a TATA box specifies Brf2 recruitment over TFIIB recruitment. Indeed, we find that it is possible to assemble efficiently a complex containing TBP, $SNAP_c$, and TFIIB on the U6 promoter. It seems likely that in human cells, so far unidentified factors associated with either TFIIB, Brf1, TBP, or $SNAP_c$ prevent assembly of TFIIB and/or favor assembly of Brf2 into the U6 initiation complex.

The second level of RNA polymerase specificity determination is protein-protein interactions among factors recruited by the promoter elements. Thus, even though the U6 promoter is capable of assembling complexes containing either TBP, $SNAP_c$, and TFIIB or TBP, $SNAP_c$, and Brf2, only the latter complex is capable of recruiting Bdp1. Moreover, the carboxy-terminal domain of Brf2 may also be involved in the recruitment of pol III. With yeast Brf1, the carboxy-terminal domain of the protein has been shown to associate with the yeast pol III subunit C34, which has no counterpart in pol II. Thus, the carboxy-terminal extensions present in the TFIIB family members involved in RNA pol III transcription, i.e., Brf1 and Brf2, are key to favoring recruitment of pol III over that of pol II because they allow recruitment of Bdp1 and, probably, of pol III itself through pol III-specific subunits.

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STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

L. Joshua-Tor	D. Chitwood	S. Smith
	E. Enemark	J.-J. Song
	H. He	T. Takara
	P.R. Kumar	N. Tolia
	P. O'Farrell	

We study the molecular basis of cell regulatory processes by using the tools of structural biology and biochemistry to examine proteins and protein complexes associated with these processes. X-ray crystallography enables us to obtain the three-dimensional structures of individual proteins and their interactions with other molecules. Biochemistry and molecular biology allow us to study properties that can be correlated with protein structure and function. Our efforts largely center on protein complexes involved in nucleic acid regulatory processes.

Mechanisms of RNAi

N. Tolia, J.-J. Song, D. Chitwood, S. Smith [in collaboration with G.J. Hannon, Cold Spring Harbor Laboratory]

The introduction of exogenous double-stranded RNA (dsRNA) into a cell can trigger the gene-silencing process called RNA interference or RNAi. An RNase III family enzyme, Dicer, initiates silencing by releasing about 20 base duplexes, with 2-nucleotide 3′ overhangs called short interfering RNAs (siRNAs). The RNAi pathway also mediates the function of endogenous, noncoding regulatory RNAs called microRNAs (miRNAs). Both miRNAs and siRNAs guide substrate selection by similar if not identical effector complexes called RISC (RNA-induced silencing complex). These contain single-stranded versions of the small RNA and additional protein components. Of those, the signature element, which virtually defines a RISC, is a member of the Argonaute family of proteins. In a central RNAi pathway, guided by the siRNA, RISC directs the cleavage of mRNA substrates. We embarked on structural and biochemical studies of key proteins in the RNAi pathway, the protein-RNA and protein-protein interactions involved. Last year, we determined the structure of a full-length Argonaute protein from *Pyrococcus furiosus* and demonstrated its similarity to RNase H. This led us to propose that Argonaute functions as the important "Slicer" in RISC and suggested a mechanism for siRNA-guided mRNA cleavage. This was tested in the mammalian system, by mutational analysis of hAgo2 as guided by our structure.

This year, we developed an expression system for hAgo2 in *Escherichia coli*. This provided an important opportunity to examine which features of RISC activity reside in Argonaute itself and which must reside elsewhere, which we have done in collaboration with Greg Hannon's lab here at CSHL. First, we learned that hAgo2 and an siRNA guide are all that is needed to carry out slicing. The 5′ phosphate of the siRNA is important for enzyme stability as well as RISC fidelity. Single turnover kinetics for this minimal enzyme are remarkably similar to those determined for RISC from either flies or human cell extracts, indicating that other factors are not needed to assist in binding and cleavage. However, an ATP dependence on turnover, characteristic of more complete RISC complexes, is absent from this minimal form, indicating that another protein assists in product release using ATP. Such a factor may trigger domain movement in Argonaute, a feature that is intimated from the various structures described above.

The DNA-binding and Assembly of the Papillomavirus Initiator Protein E1

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

Infection with the human papillomavirus (HPV) is the most common sexually transmitted disease, with an infection rate of above 40% in the college-aged population. Women with persistent infections from certain types of HPV are at risk for cervical cancer, as 99% of cervical cancers around the world are associated with

HPV infection. The papillomavirus 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 (*ori*), melt the DNA duplex, possess DNA helicase activity, and recruit other cellular replication proteins. We are pursuing structural and biochemical studies of E1, its DNA-binding activity, and its assembly on DNA. The relative simplicity of this system also allows examination of the general mechanistic features of DNA replication. Because a single protein encompasses all key events in replication initiation through different oligomeric forms, this system provides a great opportunity to examine these events on a mechanistic and atomic level. These studies can also provide a basis for the development of clinical intervention strategies.

The initial binding of an E1 dimer together with E2 serves to recognize the double-stranded *ori* with high specificity. The subsequent binding of additional E1 molecules leads to ori melting. Ultimately, two hexameric rings are formed on the ori that can unwind the DNA double helix. Thus, the transition between these complexes represents a transition from a tethering function for E1 to a function that modifies the DNA structure. 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. Initially, we solved the structure of the unbound DNA-binding domain (DBD) of E1 from BPV-1 (bovine papillomavirus type 1) and characterized the DNA-binding surfaces. We also suggested a model for binding of the initial dimer to the origin and found the dimerization surface to be essential for replication in vivo. We have also solved the structure of the DBD from a high-risk papillomavirus, HPV-18, which is associated with poor prognosis in cervical cancer patients, and characterized its DNA-binding and dimerization properties toward further studies in drug design. These DBDs are now part of a growing family of viral initiator proteins with similar structures.

More recently, we have captured structural snapshots of two sequential steps in the assembly process, with structures of both the dimeric and tetrameric forms of the E1-DBD bound to the origin DNA. We found that the mode of DNA binding employed by E1 partitions the two individual DNA strands onto distinct binding surfaces of the protein. This suggests how E1 ultimately progresses from a double-stranded origin recognition complex to a hexameric helicase where the two strands are fully separated, and each strand is encircled by a hexameric ring. We are currently studying the hexameric form of the helicase itself and its interaction with DNA.

These studies would provide general insight into the biochemical events that are involved in viral DNA replication. They can also provide 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.

Erythrocyte Invasion by the Malaria Parasite

N. Tolia [in collaboration with B.K.L. Sim, Protein Potential]

Malaria causes approximately 400 million clinical cases and two million deaths annually, with more than 80% of deaths occurring among children. The disease is caused by mosquito-borne parasites of the genus *Plasmodium* (primarily *Plasmodium falciparum*). Following the initial stages of infection, merozoite-stage parasites ("merozoites") invade red blood cells, leading to clinical symptoms and, in many cases, death.

This past year, we reported on a crystal structure of an important protein from the malaria parasite, *Plasmodium falciparum*. All of the pathological and clinical manifestations of the disease are caused by the blood (erythrocytic)-stage form of the parasite, the merozoite. Thus, disruption of the invasion of erythrocytes by merozoites might help in controlling this disease. *P. falciparum* displays a family of proteins that bind to host-cell receptors during invasion and also sequestration, where the infected blood cells bind to the capillaries of host tissues, which in turn may result in particularly severe pathologies such as cerebral malaria. Our structure, of the erythrocyte-binding domain (RII) of the erythrocyte-binding antigen, EBA-175, was solved with and without a glycan that contains the essential components of the receptor required for binding (Fig. 1). This is the first structure of this important family of proteins. The dimeric organization of RII mimics a handshake and displays two prominent channels. The RII dimer appears to grip the glycans that are bound to the dimer interface and contact both monomers. These structures provide insight into the mechanism of erythrocyte invasion by the malaria parasite (Fig. 2)

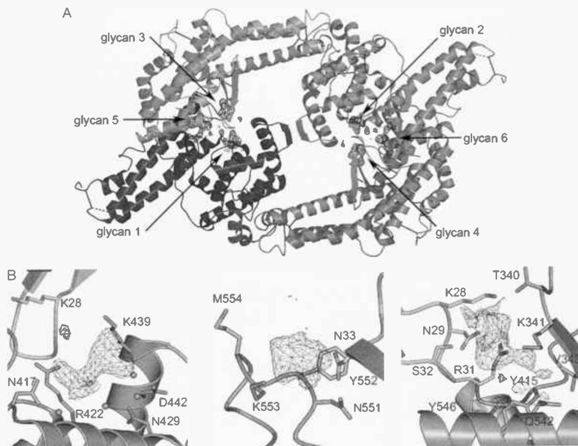

FIGURE 1 Crystal structure of RII with sialyllactose. (*A*) Ribbon representation of the dimeric structure of RII. The monomers are shaded in different intensities and are composed of two subdomains, F1 and F2. Two channels are created in the center of the molecule. Glycan positions are shown in F_o–F_c electron density. (*B*) Close-up views of three of the glycan binding sites—1, 3, and 5, from left to right. Residues from both monomers contact each glycan.

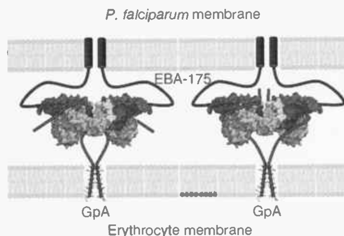

FIGURE 2 (*Top*) *P. falciparum* membrane; (*bottom*) erythrocyte membrane. The receptor-binding domain of EBA-175, RII, is shown as a surface representation. Lines above the surface represent portions of the EBA-175 backbone not included in the crystal structure. The receptor glycophorin A (GpA) is shown at the bottom with the membrane-spanning region in detail using the nuclear magnetic resonance (NMR) structure and the extracellular domain drawn as a schematic flexible line. In the left panel, the RII dimer assembles around the GpA dimer, with GpA binding within the channels. An alternative model is shown on the right, where the GpA monomers dock on the outer surface of the protein, feeding glycans into the channels.

and enable the search for small-molecule inhibitors that can block the interactions of these types of proteins to their receptors either directly or by blocking dimerization, as well as more efficient vaccine design based on these proteins.

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RNA SPLICING

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MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

RNA splicing is an essential step in the expression of most eukaryotic protein-coding genes. The spliceosome selects and pairs authentic splice sites with extremely high fidelity, which requires precise interpretation of limited and dispersed sequence information present throughout introns and exons. In humans, about 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 signaling pathways. 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 numerous protein components, as well as RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. The work in our lab focuses primarily 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. Some of the splicing factors we study have additional cellular functions, e.g., reflecting their nucleic-acid-binding properties or localization in multiple cellular compartments, and we are interested in some of these nonsplicing functions as well. A brief summary of six of our recently published studies is given below.

NOVEL *LKB1* SPLICING MUTATION ASSOCIATED WITH PEUTZ-JEGHERS SYNDROME

In collaboration with N. Resta, A. Stella, and G. Guanti (University of Bari, Italy), we characterized a novel splicing mutation associated with Peutz-Jeghers syndrome (PJS). PJS is an autosomal dominant disorder associated with gastrointestinal polyposis and increased cancer risk. PJS is caused by germ-line mutations in the tumor suppressor gene *LKB1*, which encodes a protein kinase. One familial mutation, IVS2+1A>G, alters the second intron 5′ splice site, which has sequence features of a U12-type AT-AC intron. This rare type of intron normally begins with either A or G. We found that in patients, *LKB1* RNA splicing occurs from the mutated 5′ splice site to several cryptic, noncanonical 3′ splice sites immediately adjacent to the normal 3′ splice site. In vitro splicing analysis demonstrated that this aberrant splicing is mediated by the U12-dependent spliceosome. These results indicate that the minor spliceosome can use a variety of 3′ splice site sequences to pair to a given 5′ splice site, albeit with tight constraints for maintaining the 3′ splice site position. The unusual splicing defect associated with this PJS-causing mutation uncovered interesting differences in splice site recognition between the major and minor pre-mRNA splicing pathways.

REGULATION OF hnRNP A1 SHUTTLING BY PHOSPHORYLATION

Heterogeneous nuclear ribonucleoprotein (hnRNP) A1 is an abundant RNA-binding protein involved in the regulation of alternative splicing and other cellular processes. hnRNP A1 is mainly nuclear, although it shuttles rapidly between nuclear and cytoplasmic compartments. Genotoxic stress, such as osmotic shock, activates the MKK3/6-p38 signaling pathway, which in turn results in accumulation of hnRNP A1 in the cytoplasm. This effect modulates alternative splicing regulation in vivo—by decreasing the level of nuclear hnRNP A1—and correlates with increased hnRNP A1 phosphorylation. In collaboration with J. Cáceres (Edinburgh), M. Myers (CSHL), and J. Moscat (Madrid), we have characterized the molecular mechanism involved in the cytoplasmic accumulation of

hnRNP A1 in cells subjected to osmotic shock. This treatment results in serine-specific phosphorylation within a carboxy-terminal peptide, dubbed the "F peptide," which is adjacent to the M9 motif that mediates bidirectional transport of hnRNP A1. Analysis of mutants in which the F-peptide serines were replaced by aspartic acids or alanines showed that F-peptide phosphorylation is required for the subcellular redistribution of hnRNP A1 in cells subjected to osmotic shock. Furthermore, we showed that F-peptide phosphorylation modulates the interaction of hnRNP A1 with transportin Trn1. These findings suggest that the phosphorylation of F peptide by signaling pathways regulates the rate of hnRNP A1 nuclear import.

DETERMINANTS OF 5′ SPLICE SITE STRENGTH

We previously showed that the authentic 5′ splice site of the first exon in the human β-globin gene is intrinsically stronger than a cryptic 5′ splice site located 16 nucleotides upstream. In an ongoing collaboration with R. Sachidanandam (CSHL) we have now examined by mutational analysis the contribution of individual 5′ splice site nucleotides to discrimination between these two 5′ splice sites. On the basis of the in vitro splicing efficiencies of a large panel of the wild-type and mutant substrates in two separate 5′ splice site competition assays, we established a hierarchy of 5′ splice sites and grouped them into three functional subclasses: strong, intermediate, and weak. Competition between two 5′ splice sites from different subclasses always results in selection of the 5′ splice site belonging to the stronger subclass. Moreover, each subclass has different characteristic features. Strong and intermediate 5′ splice sites can be distinguished by their predicted free energy of base pairing to the U1 small nuclear RNA (snRNA) 5′ terminus (ΔG). The extent of splicing via the strong 5′ splice sites correlates well with the ΔG, but this is not the case for competition between intermediate 5′ splice sites. Weak 5′ splice sites were used only when the competing authentic 5′ splice site was inactivated by mutation. These results indicate that extensive complementarity to U1 snRNA exerts a dominant effect for 5′ splice site selection, but in the case of competing 5′ splice sites with similarly modest complementarity to U1, the role of other 5′ splice site features is more prominent. This study reveals the importance of additional submotifs present in certain 5′ splice site sequences, whose characterization will be critical for understanding 5′ splice site selection in human genes.

GENOME-WIDE ANALYSIS OF SR PROTEIN EXONIC SPLICING ENHANCER MOTIFS

Exonic splicing enhancers (ESEs) are pre-mRNA *cis*-acting elements required for splice site recognition. In collaboration with M. Zhang here at CSHL, we previously developed a Web-based program called ESEfinder that scores any sequence for the presence of ESE motifs recognized by the human SR proteins SF2/ASF, SRp40, SRp55, and SC35 (http://rulai.cshl.edu/tools/ESE/). Using ESEfinder, we have undertaken a large-scale analysis of ESE motif distribution in human protein-coding genes. Significantly higher frequencies of ESE motifs were observed in constitutive internal protein-coding exons, compared with both their flanking intronic regions and with pseudoexons. Statistical analysis of ESE motif frequency distributions revealed a complex relationship between splice site strength and increased or decreased frequencies of particular SR protein motifs. Comparison of constitutively and alternatively spliced exons demonstrated slightly weaker splice site scores, as well as significantly fewer ESE motifs, in the alternatively spliced group. Our results underline the importance of ESE-mediated SR protein function in the process of exon definition, in the context of both constitutive splicing and regulated alternative splicing.

MECHANISMS OF EXON RECOGNITION IN THE SPINAL MUSCULAR ATROPHY GENES

Spinal muscular atrophy is a neurodegenerative genetic disorder caused by the deletion or mutation of the survival-of-motor-neuron gene, *SMN1*. An *SMN1* paralog, *SMN2*, differs by a C-to-T transition in exon 7 that causes substantial skipping of this exon, such that *SMN2* expresses only low levels of functional protein. A better understanding of *SMN* splicing mechanisms should facilitate the development of drugs that increase survival motor neuron (SMN) protein levels by improving *SMN2* exon 7 inclusion. In addition, exonic mutations that cause defective splicing give rise to many genetic diseases, and the *SMN1/2* system is a useful paradigm for understanding exon identity determinants and alternative splicing mechanisms. Skipping of *SMN2* exon 7 was previously attributed either to the loss of an SF2/ASF-dependent exonic splicing enhancer or to the creation of an hnRNP A/B-dependent exonic splicing silencer, as a result of the C-to-T transition. We extensively tested the enhancer-loss and silencer-gain models by mutagenesis, RNA interference, overexpression,

RNA splicing, and RNA-protein interaction experiments. Our results support the enhancer-loss model but also demonstrate that hnRNP A/B proteins antagonize SF2/ASF-dependent ESE activity and promote exon 7 skipping by a mechanism that is independent of the C-to-T transition and is therefore common to both *SMN1* and *SMN2*. These findings explain the basis of defective *SMN2* splicing, illustrate the fine balance between positive and negative determinants of exon identity and alternative splicing, and underscore the importance of antagonistic splicing factors and exonic elements in a disease context.

ROLE OF hnRNP A1 IN TELOMERE LENGTH REGULATION

Telomerase is a ribonucleoprotein enzyme complex that reverse-transcribes an integral RNA template to add short DNA repeats to the 3′ ends of telomeres. G-quadruplex structure in a DNA substrate can block its extension by telomerase. In collaboration with R.-M. Xu here at CSHL, we found that hnRNP A1—which was previously implicated in telomere-length regulation—binds to both single-stranded and structured human telomeric repeats, and in the latter case, it disrupts their higher-order structure, which is probably an intermolecular dimeric G quadruplex. Using an in vitro telomerase assay, we observed that depletion of hnRNP A/B proteins from HEK293 cell extracts dramatically reduced telomerase activity, which was fully recovered upon addition of purified recombinant hnRNP A1 (Fig. 1). This finding suggests that hnRNP A1 functions as an auxiliary, if not essential, factor of telomerase holoenzyme. We also showed, using chromatin immunoprecipitation, that hnRNP A1 associates with human telomeres in vivo. We proposed that hnRNP A1 stimulates telomere elongation through unwinding of a G quadruplex or G-G hairpin structure formed at each translocation step, thereby enhancing the repeat-addition processivity of telomerase.

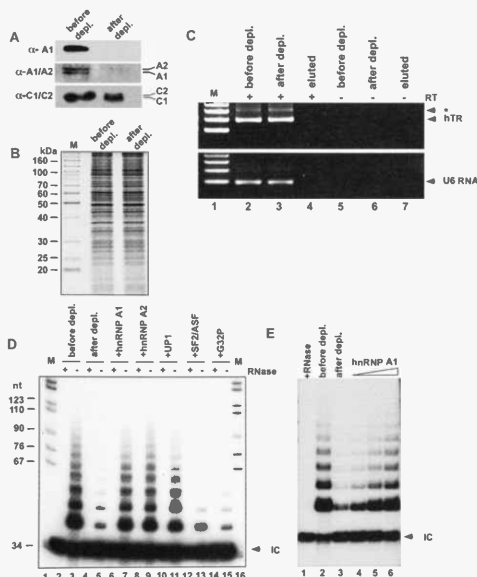

FIGURE 1 Effect of depletion and add-back of hnRNP A/B proteins on telomerase activity. Telomerase extracts prepared from HEK293 cells were incubated with immobilized, 5′-biotinylated single-stranded DNA with three human telomeric repeats, $(TTAGGG)_3$, to generate extracts depleted of components that bind tightly to these repeats. (*A*) Western blot showing the successful depletion of hnRNP A1 and A2 from the telomerase extracts. Monoclonal antibodies were used for detection of hnRNP A1, hnRNP A1/A2, and hnRNP C1/C2. (*B*) Silver staining of telomerase extracts before and after depletion of hnRNP A/B proteins. M, markers (sizes indicated at left). (*C*) RT-PCR detection of telomerase RNA (hTR) before and after depletion of hnRNP A/B proteins. RNA was purified from the extracts and from the material bound to the beads, and hTR and control U6 RNAs were detected by RT-PCR (lanes *2–4*). Control reactions without reverse transcriptase are also shown (lanes *5–7*). The PCR products corresponding to hTR and U6 snRNA are indicated. The asterisk denotes an unknown product that was also amplified with the hTR primers. (*D*) Telomerase activity in the presence and absence of hnRNP A/B proteins. The indicated recombinant proteins (5 pmoles) were added to the depleted extract individually. For every condition, RNase A was added as a control in the indicated lanes to destroy the telomerase RNA. Telomerase activity was measured by TRAP (telomeric repeat amplification protocol). (M) DNA markers (sizes indicated at left); (IC) internal control PCR product. (*E*) Titration of recombinant hnRNP A1 to show the concentration dependence of telomerase stimulatory activity. The amounts of hnRNP A1 used for lanes *4–6* were 0.16, 0.63, and 2.5 pmoles, respectively.

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Rahul Sinha

PROTEOMICS

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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. Our laboratory is focused 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, because the sample preparation becomes increasingly important to ensure success. We have been optimizing a number of parameters to increase this success rate.

As part of the optimization, we have found that nanoscale chromatography gives the best sensitivity. Using conventional 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. 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). Although these mesoporous materials offer significant advantages when analyzing relatively simple mixtures (a few hundred components), we have found them to be unsuitable for use for the analysis of complex mixtures (greater than 1000 components). This is largely due to the greater peak capacity of beaded chromatography media. We are currently trying to imbed beaded media into the mesoporous material to produce a composite material that has the benefits of both materials.

In addition to improving the chromatographic separation, we have been attempting to improve the sample preparation before chromatographic separation. In the past, we relied on small reverse-phase columns for sample cleanup and concentration. However, these columns are difficult to customize to sample amount, sample volume, and optimal type of purification required (ion exchange, reverse phase, normal phase, HILIC, IMAC, etc.). Therefore, we have focused on developing a microbead-based sample preparation strategy. We can customize the amount of microbeads and the time of binding to suit the scale of purification needed and we can change the type of purification by changing the chemistry on the microbeads. We have found that for most analyses, the microbead format performs as well as our current microcolumn format. Importantly, the microbead format also increases the throughput of sample prep, allowing many more samples to be processed simultaneously, and we are currently attempting to place the microbeads directly in line with nanoscale chromatography. The in-line analysis is expected to result in a significant increase in sensitivity and throughput, especially in cases requiring complex, multidimensional (i.e., MuDPIT) analysis.

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. Phophorylation 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 phosphorylation 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 mass spectrometry, (2) phosphorylation is generally substoichiometric, and (3) phosphorylated residues readily undergo neutral loss of the phosphoryl group. We have expended a significant effort exploring why phosphorylation suppresses peptide signals during MS. Conventional wisdom dictates that the poor signal of phosphopeptides was due solely to their strong negative charge. Our analysis indicated that this is not the case. For example, phosphorylated peptides are equally suppressed regardless of whether positive or negative ions are detected, and sulfated peptides (which carry a similar strong negative charge) do not exhibit the same suppression as their phosphorylated cousins. Our results suggest that the suppression of phosphopeptides is likely the result of a combination of the poor volatility of the phosphoryl group and/or instability of the phosphopeptides in the gas phase. We have performed an exhaustive search for conditions that alleviate this suppression and have found that switching the MALDI (matrix-assisted laser desorption ionization) matrix provides a significant improvement in the detection of phosphorylated peptides. The most commonly used matrix compounds, CHCA and DHB typically result in a 10- to 100-fold suppression of signal from phosphorylated peptides. However, *trans*-3-indole acrylic acid only results in an approximately 1.5-fold reduction in signal from phosphorylated peptides. Importantly, 3-indole acrylic acid is typically used for the analysis of poorly volatile, nonpolar compounds, which likely accounts for why 3-indole acrylic acid alleviates the suppression usually seen in the analysis of phosphorylated peptides.

"Small World" Proteomics

R. Bish, C. van der Meijden [in collaboration with
R. Sachidanandam, 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, because 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 to genetic alterations, such as loss of a node (gene) that occurs during tumorigenesis. The large number of essential genes, 15–20%, depending on the organism, suggests that the network is very intolerant of gene loss. Interestingly, we have found that the yeast nonessential and essential genes differ in their chromosomal distributions, indicative of a selective pressure excluding essential genes from some areas and enriching them in others. In contrast to normal proteome networks, the cancer network is expected to be robust to gene loss, largely because genomic instability is one of the hallmarks of human cancer.

We were hoping that our analysis of the yeast proteome network would reveal properties of mammalian networks and perhaps even how these networks become 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 global approaches would result in few inroads. Therefore, we have focused on studying the DNA damage response, partially due to our finding on the distribution of essential genes in yeast and partially because most tumors are initially sensitive to therapies based on DNA damaging agents.

DNA damage elicits a signaling cascade that results in cell cycle arrest and recruitment of the DNA repair machinery to sites of DNA damage. Importantly, polyubiquitin chains have an important role in this recruitment. In fact, *BRCA1*, a gene identified in hereditary forms of breast cancer, is a critical factor for DNA repair and functions as an ubiquitin ligase. Interestingly, *BRCA1* elaborates a specific form of polyubiquitin, K6-linked chains. However, the substrates and effectors of this chain are unclear, and we attempted to identify potential *BRCA1* effectors by identifying proteins that are associated with K6-linked polyubiquitin chains. This resulted in the identification of a WHIP (*W*erner's *h*elicase *i*nteracting *p*rotein). Importantly, WHIP binds to polyubiquitin via a previously uncharacterized polyubiquitin-binding domain. The yeast homolog of WHIP, MGS1, has previously been implicated in DNA repair, and we have found WHIP to be in a larger network of DNA repair proteins, including Werner's helicase, ERCC1, ERCC4, and a human RuvB homolog. In addition, we also find WHIP to be associated with proteins involved in the unfolded protein response, such as CHIP and Bag2. These findings suggest that WHIP may function in both DNA damage repair and as a ubiquitin-dependent chaperone. Interestingly, RAD23 also has a dual role in DNA damage repair and as a ubiquitin chaperone, suggesting a heretofore, unrecognized connection between these two pathways.

We have also been analyzing mammalian homologs of the yeast CCR4 protein. Importantly, yeast CCR4 plays an important cell cycle checkpoint function in response both to DNA damage and metabolic state. Mammalian systems contain at least three proteins with significant homology to yCCR4 and include CCR4, TTRAP, and nocturnin. Importantly, nocturnin has been implicated in linking the cell cycle to circadian rhythm. It is likely that this linkage involves the ability of CCR4 family members to function as a metabolic checkpoint of the cell cycle. Importantly, this checkpoint appears to be nonfunctional in most human tumors.

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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 main focus of our research is to understand the functions of accessory proteins Nef and Vpr of human and simian immunodeficiency viruses (HIV and SIV). These proteins are important determinants of virulence. We directed a major effort toward the identification of mechanisms and downstream effectors that mediate the effects of Nef and Vpr on cell machineries. Our experiments have been concentrated in four areas: First, 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. 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. Second, we continued 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. Third, we continued to address the relevance of individual Nef activities in vivo. To this end, we characterized the impact of Nef interaction with one of its targets, the AP-2 clathrin adapter complex, on lentivirus replication and progression to AIDS in an SIV-infected rhesus monkey model of human AIDS. Finally, we initiated experiments aimed at purifying and identifying downstream effectors of the lentiviral Vpr/Vpx family of accessory proteins. These experiments have led to the identification of several novel cellular proteins that tightly associate with Vpr/Vpx in monocytes. Among them are components of protein complexes involved with DNA damage repair and proteolysis. Current studies are aimed at verifying these novel interactions and addressing their relevance to previously known and possibly novel functions of Vpr/Vpx lentiviral accessory factors.

NEF AND CHEMOTACTIC MACHINERY IN T LYMPHOCYTES

In our initial studies, we noticed that CXCR4 levels on the surface of cells expressing HIV-1 Nef are slightly decreased. CXCR4 is a receptor for the stroma-derived factor 1 (SDF-1) chemokine and is essential for lymphocyte migration to SDF-1. CXCR4 is also an entry cofactor for HIV-1 but not HIV-2 or SIV viruses. These observations suggested that HIV-1 may down-regulate cell surface expression of CXCR4 and that this effect could be relevant to HIV-1 entry into the target cells. Alternatively, down-regulating CXCR4 expression could provide an additional mechanism for Nef to inhibit T-cell migration to the SDF-1 chemokine and have another entry-independent role in the leniviral life cycle. To address these possibilities, we examined the effects of Nef proteins from diverse groups of primate lentiviruses including HIV-1 and HIV-2 and SIVmac239, SIVsm, and SIVagm on CXCR4 cell surface expression.

Flow cytometric analysis of T lymphocytes expressing SIVmac239, SIVsm, and SIVagm Nef proteins revealed that they strongly down-modulate cell surface expression of CXCR4. Notably, these viruses generally do not utilize the CXCR4 chemokine receptor for entry into target cells. In contrast, all HIV-1 and the majority of HIV-2 Nef proteins tested did not have such strong effects. Nef is known to down-modulate several cell surface receptors that are crucial for normal immune function by altering their trafficking in the cell. Therefore, we asked whether Nef also disrupts CXCR4 trafficking. The experiments revealed that low-level cell surface CXCR4 expression correlated with accelerated CXCR4 internalization from the cell surface and thus indicated that Nef proteins also induce CXCR4 endocytosis. Notably, CXCR4 down-modulation was abolished by mutations that disrupt the constitutively strong AP-2-binding element locat-

ed in the amino-terminal region of the SIV Nef molecule, suggesting that Nef induces CXCR4 endocytosis via the AP-2 clathrin adapter pathway. Together, these results point to CXCR4 having an important role for SIV persistence in vivo that is unrelated to viral entry into the target cells. Rather, Nef targets CXCR4 to disrupt ordered trafficking of the infected leukocytes between local microenvironments. This in turn could facilitate their dissemination and/or impair antiviral immune response.

NOVEL DOWNSTREAM EFFECTORS OF LENTIVIRAL NEF PROTEINS

We completed biochemical/proteomic experiments designed to purify and identify by mass spectroscopy cellular proteins to which Nef binds. Specifically, we produced gram quantities of Jurkat T lymphoblasts and U937 monocytes expressing hemagglutinin (HA)- and FLAG-epitope-tagged SIVmac and HIV-1 Nef proteins and purified Nef and their associated cellular proteins by two sequential immunoprecipitations with anti-HA and anti-FLAG monoclonal antibodies, each followed by competitive elution with the appropriate peptide. We next collaborated with Drs. M. Washburn and L. Florens (Stowers Institute for Medical Research, Kansas City, Kansas) who performed mass spectroscopic identification of Nef-associated cellular proteins using multidimensional protein identification technology (MudPIT). The MudPIT approach identified several novel and potentially relevant interactions between Nef and cellular proteins. First, we found that in U937 cells, Nef binds ELMO2. ELMO2 is an isoform of ELMO1, a subunit of the DOCK2-ELMO1 activator of the small Rho family GTPase Rac that we previously showed to be targeted by Nef in T lymphocytes. This observation indicates that Nef targets not only specifically the DOCK2 and ELMO1 isoforms, but also other members of these protein families and furthermore suggests that Nef could deregulate Rac activity through this mechanism in a wider range of cell types than previously suspected. This latter possibility will be addressed in more detail in future studies. Second, we found that both the HIV-1 and SIVmac Nef bind specifically common cellular proteins such as, for example, PCMT1 and LETM1 in both Jurkat and U937 cells. PCMT1 is a protein "repair" enzyme. It initiates the conversion of damaged L-isoaspartyl and D-aspartyl residues to normal L-aspartyl residues. The "repair" reaction helps to maintain proper protein conformation by preventing the accumulation of damaged proteins containing abnormal amino acid residues. LETM1 is an inner mitochondrial membrane protein that regulates the potassium ion gradient and membrane potential across the mitochondrion membrane and likely has a crucial role for energy metabolism by mitochondria. Thus, both LETM1 and PCMT1 could be important and relevant targets for Nef in cells infected by primate lentiviruses. We are now assessing whether Nef modulates the normal functions of PCMT1 and/or LETM1, as well as selected mitochondrial functions.

PURIFICATION AND IDENTIFICATION BY MASS SPECTROSCOPY OF NOVEL PUTATIVE EFFECTORS OF LENTIVIRAL VPR PROTEINS

Vpr is one of the accessory proteins of HIV-1. One function of HIV-1 Vpr is to mediate translocation of viral reverse transcription complexes into the nucleus in nondividing cells, such as terminally differentiated macrophages. The other effect is to perturb the cell cycle progression and proliferation status of the infected cell. Both functions are thought to be important for facilitating the HIV-1 life cycle in the infected host. Unlike HIV-1, HIV-2/SIVmac viruses encode in addition to Vpr a closely related protein called Vpx. Notably, SIV Vpr blocks cell cycle progression in the G_2/M phase, similar to Vpr proteins from HIV-1 strains, but it does not have the ability to promote nuclear transport of the preintegration complexes in nondividing cells. In contrast, Vpx mediates the nuclear import of the viral reverse transcription complex, but it does not affect cell cycle progression of the host cell. Thus, two independent functions of the HIV-1 Vpr protein are encoded by separate accessory proteins in HIV-2/SIV viruses. We thought that comparative studies of Vpr/Vpx would provide a powerful system with which to analyze the molecular mechanisms mediating the cell cycle and nuclear transport effects of these proteins.

As a first step toward this goal, we set up to identify cellular proteins that Vpr and Vpx associate with in the cell. The unbiased approaches that have been used so far to identify immediate downstream effectors of Vpr proteins have relied heavily on the abilities to reconstruct the relevant interactions in heterologous or in in vitro systems, such as the yeast two-hybrid (YTH) interaction screen. These approaches have correctly identified a subset of Vpr ligands, but they have their limitations and have been used exhaustively. Therefore, to bypass the limitations of the previously used meth-

ods, we purified to near homogeneity HIV-1 Vpr, SIVmac Vpr, and SIV Vpx and their associated protein complexes from stably transfected U937 monocyte/macrophage as well as transiently transfected HEK 293T cells, using an immunoaffinity purification protocol. With this approach, we detected a series of polypeptides with apparent molecular masses ranging from 20 kD to more than 250 kD that copurified with HIV-1 and SIV Vpr/Vpx proteins from both cell lines. Next, we determined their sequences by mass spectroscopic analysis, in collaboration with Dr. M. Washburn and L. Florens (Stowers Institute for Medical Research, Kansas City, Kansas). This led to the identification of several common polypeptides associated with Vpr and Vpx proteins. We are currently characterizing the selected interactions and their effects on lentiviral life cycle and normal cell functions.

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Smita Srivastava

TRANSCRIPTIONAL CONTROL AND THE UBIQUITIN-PROTEASOME SYSTEM

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Ubiquitin (Ub)-mediated proteolysis is a process in which the covalent attachment of Ub to target proteins signals their destruction by the 26S proteasome. The controlled destruction of proteins by this system is used to fine-tune the levels of proteins within the cell, allows cells to rapidly alter their protein composition in response to particular signals, and provides rigorous control and directionality to processes such as the cell cycle. Our particular interest centers on how Ub-mediated proteolysis regulates gene transcription. In recent years, a body of evidence has accumulated suggesting that Ub, the proteasome, and other components of the Ub system are involved—directly and mechanistically—in the regulation of gene activity. We are interested in understanding how the Ub system regulates transcription and the consequences of this regulation for the control of cell growth in normal and cancer cells.

Research in our laboratory is divided in two areas. To study the basic processes that connect the transcription and Ub systems, we perform biochemical and genetic experiments using the brewer's yeast *Saccharomyces cerevisiae* as a model. To understand the biological significance of the intersections between these systems, we study the human oncoprotein transcription factor Myc.

REGULATION OF MYC BY UBIQUITIN-MEDIATED PROTEOLYSIS

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 features in approximately 70,000 cancer deaths in the United States each year.

Like many transcription factors, particularly those involved in the control of cell growth, Myc is an unstable protein that is destroyed by Ub-mediated proteolysis. Previous work in our laboratory has revealed that the destruction of transcription factors such as Myc 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 ubiquitin-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. Thus, we believe that by probing the mechanisms through which Myc is destroyed by the Ub system—the elements involved, the cellular players that control Myc turnover, and the pathways that govern Myc stability—we can also gain new insight into how this enigmatic human oncoprotein functions.

For example, we and others have found that adenovirus E1A can stabilize Myc during the course of adenovirus infection. E1A is a prototypical viral oncoprotein that functions to regulate the expression of both viral and cellular genes. The analysis of E1A has been instrumental in revealing critical pathways that lead to cellular transformation. Although the significance of E1A-mediated stabilization of Myc was not known, the intimate connection between transcription factor activity and destruction led us to speculate that this stabilization *must* have a functional consequence. In collaboration with the Hannon and Lowe labs here at CSHL, we therefore investigated the mechanism through which E1A stabilizes Myc and probed the relationship of this mechanism to the function of the E1A oncoprotein. We found that 12S E1A, when sta-

bly expressed in U2OS cells or in nontransformed IMR90 human diploid fibroblasts, promoted the accumulation of Myc protein. We subsequently showed that E1A expression stabilizes Myc, without affecting its ubiquitylation, and that it does so via the transcriptional coactivator p400. This was an interesting finding, because although the E1A-p400 interaction was known to be important for E1A function, the downstream target of this interaction was unknown. The functional significance of the E1A-p400-Myc nexus was demonstrated by (1) the observation that Myc, like p400, is essential for E1A to induce apoptosis, (2) the finding that E1A can stimulate expression of Myc target genes, and (3) the finding that in vitro transformation defects that arise upon disruption of the p400-binding site in E1A can be ameliorated by overexpression of Myc, arguing that the essential function of this region of E1A is to increase Myc protein levels. Taken together, these data reveal that interaction of E1A with p400 leads to an increase in the levels of p400, which in turn results in a postubiquitylation stabilization of Myc and a subsequent activation of Myc target genes. In essence, E1A (via p400) "hijacks" Myc to perform an important facet of its activities. We are excited to learn about this novel pathway of E1A function and keen to explore the mechanism through which E1A regulates p400 and through which p400 modulates Myc.

In addition to understanding how the factors that control Myc stability impact Myc activity, we are interested in learning how the elements within Myc that signal its destruction participate in transcriptional regulation. The transcription activation domain (TAD) of Myc spans the amino-terminal 143 residues and includes two highly conserved regions known as Myc Box (MB) I and MBII. Myc boxes have been implicated in both transcriptional regulation and proteolysis, lending support to the concept that proteolytic control of Myc is tied to both its conservation and its activity as a transcription factor. Several years ago, we performed a systematic deletion analysis of Myc and identified a second destruction element within Myc we called the "D-element." Interestingly, the D-element overlaps with another highly conserved sequence—Myc Box III (MBIII). MBIII was originally identified about 20 years ago, but it has not been studied extensively, leaving a considerable gap in our knowledge about Myc function. Because Myc boxes have been shown to be involved in both the activity and destruction of Myc, we revisited the roles of these conserved elements in various aspects of Myc biology. To accomplish this goal, we have compared the activities of wild-type versus MB-deleted Myc proteins. One of the most intriguing findings of this work is that transcriptional repression by Myc is largely dependent on MBIII. Transcriptional repression by Myc is an important and understudied topic and is thought to have a key role in Myc-driven entry into the S phase of the cell cycle. As such, the transcriptional regulation of key factors such as p15 and p21 by Myc could be an important component of Myc's ability to cause cancer, and we have recently focused on identifying the mechanisms by which Myc, through MBIII, represses these and other Myc targets. Our studies to date demonstrate that MBIII can function by altering the pattern of covalent histone modifications at Myc target genes, thereby impeding access of the general transcriptional machinery to sites of Myc target gene transcription in vivo. Our studies in this area not only reinforce the concept that proteolysis can be used as a platform to study transcription factor activity, but provide novel insight into how Myc, through its conserved elements and recruitment of transcriptional regulators, chromatin modifiers, and novel interacting proteins, controls gene expression.

THE ROLE OF THE UBIQUITIN-PROTEASOME SYSTEM IN GENE ACTIVITY

Our original observation of the tight relationship between transcriptional activation and Ub-mediated destruction of transcriptional activators predicted that, at some level, components of the Ub-proteasome system would be involved in transcriptional regulation. During the past several years, work from a number of groups, including our own, has supported this prediction. For example, we have found that Ub ligases can be recruited to sites of transcription, where they perform essential steps in gene activation, including driving postinitiation changes in the entourage of RNA-polymerase-interacting proteins. Other groups have found that Ub ligases can promote coactivator exchange at promoters in vivo, as well as targeting stalled RNA polymerases for destruction to clear the path for successful gene transcription following DNA-damage-mediated transcriptional arrest. Moreover, ubiquitylation of histones has been linked to transcriptional repression and shown to participate in the regulation of covalent patterns of histone modifications. Our ongoing studies in this area continue to focus on the role of activator ubiquitylation, but we are also studying ubiquitylation of RNA polymerase, histones,

and the relationship between the Ub system and pre-mRNA processing.

In addition to our interest in Ub-dependent *trans*-actions and how they feature in transcription, we are also interesting in the role of the proteasome in regulating gene activity. Inspired by work from Stephen Johnston's laboratory at Arizona State University, we have also found that components of the proteasome are recruited to sites of transcription in yeast cells. Recruitment of proteasome components to chromatin depends on the monoubiquitylation of histone H2B and is important for multiple steps in the transcription process, including the transcription-coupled methylation of histone H3. Curiously, we have also found that proteasome subunits associate with gene promoters before activation, suggesting that these proteins are involved in either maintaining genes in the inactive state or in some very early step in transcriptional activation. In collaboration with the laboratory of Jerry Workman at Stowers Institute, we demonstrated that at the *GAL* genes in yeast, the latter situation is likely. We found that mutations in ATPase subunits of the 19S proteasome specifically give rise to defects at the earliest stage of transcriptional activation of the *GAL10* gene: recruitment of the SAGA chromatin remodeling complex. In vitro studies supported this observation by showing that 19S proteins, in the absence of 20S components of the proteasome, could facilitate the stable interaction between a DNA-bound Gal4 activator and the SAGA complex. This effect was most pronounced at limiting levels of SAGA and required both intact ATPase function of the 19S complex and ATP hydrolysis. The ability of the 19S complex to stabilize interactions between an activator and its target in the transcriptional machinery is very similar to the role of loading factors in DNA replication, which use the energy of ATP hydrolysis to drive chaperone-like activities that direct the specific and ordered assembly of pre-replication complexes at origins of replication. By analogy, we therefore posit that the 19S proteins use their chaperone functions to act as loading factors for gene transcription. We suggest that the localization of 19S proteins to promoters prior to activation not only serves to coordinate early events in the activation process, but provides a high level of specificity to the process; if the activator and SAGA can only stably associate in the presence of 19S proteins, and if 19S proteins are localized to promoters (which appears to be the case prior to transcription), then the activator and SAGA will only come together on promoter DNAs in response to the correct signals. Ongoing studies in this area are directed toward understanding exactly how these 19S proteins function, identifying their targets in the transcriptional machinery, and probing whether 19S and other subunits of the proteasome are involved in additional steps in the transcription process.

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STRUCTURAL BIOLOGY

R.-M. Xu H.-C. Hsu Z. Li
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We continued to focus our research on structural studies of proteins involved in mRNA processing and proteins that are important for the assembly and remodeling of higher-order chromatin structures. During 2005, we have determined the structures of a human Bmi1-Ring1B oncoprotein complex; a complex of EMSY, a BRCA2-associated protein, and the chromo shadow domain (CSD) of HP1; and a structure of a methylated histone H3 peptide in complex with the double-tudor domain of a Jmjc domain histone demethylase, JMJD2A. Our structural results provide important insights into molecular mechanisms by which these proteins function in modulating higher-order chromatin structure and oncogenesis.

BMI1-RING1B POLYCOMB GROUP UBIQUITIN LIGASE COMPLEX

Polycomb group (PcG) proteins are a set of evolutionarily conserved transcriptional repressors controlling homeotic gene expression during development. Biochemical and genetic characterizations of PcG proteins have revealed that they exist in distinct complexes, of which the two best characterized are the PRC1 and PRC2 complexes. Both PcG complexes have been implicated in diverse biological processes such as epigenetic inheritance, stem-cell development, senescence, and tumorigenesis. A human PRC1 complex composed of Bmi-1, HPH2, PC3, and Ring proteins (Ring1A and Ring1B), which are homologs of *Drosophila* Psc, Ph, Pc, and dRing, respectively, is an E3 ubiquitin ligase complex that mono-ubiquitinates Lys-119 of nucleosomal histone H2A. The catalytic subunit of the PRC1 E3 ligase complex is Ring1B. The E3 ligase activity has been shown to be important for the involvement of PRC1 in X-chromosome inactivation and the control of *Hox* gene expression. Bmi-1, a *Drosophila* Psc homolog that was originally discovered through its ability to collaborate with Myc in lymphomagenesis, has a central role in the assembly of the PRC1 complex, and although Bmi-1 displays no detectable ubiquitin ligase activity, the binding of Bmi-1 greatly stimulates the E3 ligase activity of Ring1B.

We have mapped the regions of Bmi-1 and Ring1B required for efficient ubiquitin transfer and determined a 2.5-Å structure of the Bmi-1–Ring1B core domain complex. Both proteins contain a canonical RING domain, which is composed of a three-stranded antiparallel β-sheet in the 2↑1↓3↑ configuration, two zinc-binding loops, and an α helix immediately carboxy-terminal to β2. Ring1B "hugs" Bmi-1 through extensive RING domain contacts, and its amino-terminal tail wraps around Bmi-1. The two RING domains are positioned in a manner similar to that of the only other known heterodimeric RING domain structure, the BRCA1-BARD1 RING-RING complex. We also showed that the two regions of interaction have a synergistic effect on the E3 ligase activity. Our analyses suggest a model where the Bmi-1–Ring1B complex stabilizes the interaction between the E2 enzyme and the nucleosomal substrate to allow efficient ubiquitin transfer.

EMSY-HP1 INTERACTION

Heterochromatin protein-1 (HP1) has an essential role in both the assembly of higher-order chromatin structure and epigenetic inheritance. Various proteins important for chromatin structure and gene transcription have been found to interact with the HP1 CSD. They include the large subunit of chromatin assembly factor-1 (CAF-1) and components of the cellular transcriptional machinery. In addition, the largest subunit of the origin recognition complex (Orc1) interacts with full-length HP1, rather than individual domains. A recent addition to the list of HP1-interacting proteins is EMSY, which was identified as a protein that interacts with the exon-3-encoded transcriptional activation domain of breast cancer susceptibility gene 2 (*BRCA2*). EMSY has been implicated in *BRCA2*-associated sporadic breast and ovarian cancer.

The EMSY sequence implicated in HP1-binding, RLVPL, deviates from the consensus HP1 binding sequence PXVXL (positions –2 to +2) at the –2 position. In the structure of the HP1 CSD-CAF-1 peptide complex, the proline at position –2 occupies a shallow hydrophobic pocket, which an arginine would not be

able to bind. To determine the exact mode of interaction between the HP1 CSD and EMSY, we have determined the crystal structure of the HP1β CSD in complex with the amino-terminal domain of EMSY at 1.8-Å resolution. Surprisingly, the structure reveals that EMSY is bound by two HP1 CSD homodimers, and the binding sequences, LVPLM and FTVTA, differ from the consensus HP1-binding motif PXVXL. This structural information expands our understanding of HP1-binding specificity and provides insights into interactions between HP1 homodimers that are likely to be important for heterochromatin formation.

RECOGNITION OF METHYLATED HISTONE TAILS BY THE DOUBLE-TUDOR DOMAIN OF JMJD2A

Biological responses to histone methylation critically depend on the faithful readout and transduction of the methyl-lysine signal by "effector" proteins, yet our understanding of methyl-lysine recognition has so far been limited to the study of histone binding by chromodomain and WD40-repeat proteins. The double-tudor domain of JMJD2A, a Jmjc-domain-containing histone demethylase, binds methylated histone H3-K4 and H4-K20. We found that the double-tudor domain has an interdigitated structure, and the unusual fold is required for its ability to bind methylated histone tails. The cocrystal structure of the JMJD2A double-tudor domain with a trimethylated H3-K4 peptide reveals that the trimethyl-K4 is bound in a cage of three aromatic residues, two of which are from the tudor-2 motif, whereas the binding specificity is determined by side-chain interactions involving amino acids from the tudor-1 motif. Our study provides mechanistic insights into recognition of methylated histone tails by tudor domains and reveals the structural intricacy of methyl-lysine recognition by two closely spaced effector domains.

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CANCER: GENETICS

In some cases, the fusion of human cells is a normal process that leads, for instance, to the formation of muscle and bone. Seemingly innocuous infections by common viruses can also cause cells in our bodies to fuse. Such fused cells are widely considered to be harmless because they are generally believed to die and be cleared from the body without consequences for our health. This view of cells fused by viruses as being harmless may need to be revised. A recent study of cultured cells by Yuri Lazebnik and his colleagues has revealed that cell fusion triggered by common viral infections may be a significant factor in the development of human cancer. Lazebnik's group is currently testing this possibility in animal models. Non-CSHL scientists are developing fusogenic viruses as gene delivery tools for human gene therapy. Lazebnik's research indicates that because these viruses might cause cancer, their use for therapeutic purposes should be carefully evaluated.

It is becoming clear that cancer and aging are inextricably intertwined by molecular pathways that link the two processes. Alea Mills is using a variety of mouse models that enable her to investigate these links. Specifically, she is interested in p63, a protein similar to the well-known tumor suppressor protein p53. Mills's recent experiments have demonstrated that loss of the *p63* gene accelerates aging in mice. She is now investigating p63's role in the delicate balance between aging and cancer, work that may lead to anti-cancer therapies.

A desire to understand why nearly all cancers contain multiple copies of some portions of chromosomes drove investigators Michael Wigler and Rob Lucito to develop a powerful method for measuring the difference in DNA copy number between normal and cancer cells. Using their method, called representational oligonucleotide microarray analysis, or ROMA, they have identified chromosomal regions that had not previously been known to have a role in cancer. Some of these regions are common to both early and advanced cancers, and others are seen predominantly in advanced disease. A particular hallmark of lethal cancers is a type of lesion Wigler has dubbed "firestorms" to describe highly unstable chromosomal regions. Through the sort of "DNA biopsy" that the ROMA technology provides, the researchers are aiming to improve the way all major forms of cancer are diagnosed and treated. Lucito is also modifying ROMA so it can be used to analyze modifications to DNA, so-called epigenetic changes, that often accompany cancer.

Jonathan Sebat is using ROMA to test the idea that changes in gene-copy number contribute to sporadic cases of autism. An initial study of 110 sporadic cases of autism identified six spontaneous mutations, five of which have been previously reported in association with autism. The sixth variant is a deletion on chromosome 20 that contains multiple genes potentially involved in human development, particularly of the central nervous system. Locating this new autism-associated region could help researchers identify the underlying genetic changes that lead to autism.

Eli Hatchwell is also interested in mutations that arise spontaneously and cause sporadic cases of disease. Because spontaneous mutations are typically rare and are usually not inherited, traditional methods of locating such mutations by studying related individuals are of no use. However, spontaneous mutations can provide invaluable clues to the origin of common disorders. For example, the identification of spontaneous deletions in chromosome 22 have been linked to schizophrenia. Hatchwell has developed a method to evaluate large sections of the genome for microdeletions, a type of genetic instability too small to be identified by karyotyping and other traditional approaches. By using this method, he has located genes associated with neurological disorders such as autism and the rare but informative Toriello-Carey syndrome.

RNA interference (RNAi) is a fundamental molecular process that controls gene expression in many organisms. CSHL scientists understood the power of RNAi early on and continue to make many seminal discoveries about it. Moreover, they are putting RNAi to several uses in both basic and applied research. One such development—the creation of libraries of short interfering RNAs targeted to each gene in the human and mouse genome—was completed this year by Greg Hannon's group in collaboration with

investigators at Harvard, Merck, and OSI Pharmaceuticals. Hannon, one of the first scientists to investigate how RNAi controls gene expression, continues to explore the components, mechanisms, and applications of this powerful gene regulatory system. In collaboration with Scott Lowe, Scott Powers, and CSHL alumnus Scott Hammond, Hannon and his colleagues discovered that a particular noncoding "microRNA" has a role—through RNAi—in human cancer. Another investigation by Hannon's group of the RNAi components in mice led to the discovery of an entirely new class of small RNAs that, interestingly, are found only in males.

Vivek Mittal studies the mechanism by which tumors spur their own growth by creating new blood vessels to feed themselves. This year, his group established that blood vessel progenitor cells come from the bone marrow and travel to the tumor site, where they respond to growth signals and form new blood vessels that nourish the growing tumor. Mittal has shown that signals from the tumor activate particular pathways within the blood vessel progenitor cells. Experiments using RNAi to knock down the levels of these signals markedly delays tumor growth and reveals a potential new target for cancer therapy.

Masaaki Hamaguchi (now at Fordham University) studies a tumor suppressor gene he discovered at CSHL called *DBC2* (deleted in breast cancer 2), which he showed is linked to breast and lung cancer. This year, by using RNAi, Hamaguchi identified some 680 genes whose activity is altered when the Dbc2 protein is decreased as it is in tumors. His analysis of the isolated genes led to the discovery of four major cellular networks in which Dbc2 has a significant impact. Further investigation revealed that Dbc2 is critical for proper protein processing and transport.

ANALYSIS OF A TUMOR SUPPRESSOR GENE

M. Hamaguchi F.K. Chang
N. Kobayashi
J.L. Meth

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. We are characterizing *DBC2* (*d*eleted in *b*reast *c*ancer 2), which 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. We have discovered that *DBC2* demonstrates pleiotropism by expression profiling analysis. We further investigated each biological pathway suggested to involve *DBC2*. The ultimate goal of our research is the elucidation of tumor suppression mechanisms of *DBC2* and development of new cancer therapy based on the findings.

DBC2 and Gene Expression

M. Hamaguchi, N. Kobayashi, J.L. Meth

To clarify *DBC2*'s physiological functions, we used microarray analysis to isolate approximately 700 genes that were disturbed by altered *DBC2* expression. The gene list was overlaid onto a huge cellular pathway map in a database so that we could identify biological pathways influenced by *DBC2*. We discovered four cellular pathways that are significantly affected by manipulation of *DBC2* function. They are related to apoptosis, cell cycle control, membrane trafficking, and cytoskeleton. This discovery is particularly important because a gene family, RHOBTB, where *DBC2* belongs, has not been characterized; it would provide a framework for functional studies of this potentially important gene family.

DBC2 and Protein Transport

M. Hamaguchi, F.K. Chang

As part of comprehensive research on *DBC2* function, we studied its role in protein transport, using the VSVG-ts045-GFP (ts045) protein as a reporter of membrane trafficking. This visible protein is transported from the endoplasmic reticulum (ER) to the cellular membrane via the Golgi apparatus in a microtubule-dependent manner. We discovered that when *DBC2* is knocked down by RNA interference (RNAi), ts045 transport is hindered in certain cells, including 293 and HeLa cells that express *DBC2* under physiological conditions. We further demonstrated that *DBC2* movement also depends on the microtubule network. We are currently testing the hypothesis that *DBC2* has a role in connecting vesicles (vehicles for protein transport) and the cellular motor system.

DBC2 and Cell Cycle Control

M. Hamaguchi, N. Kobayashi

We discovered that *DBC2* negatively regulates the CCND1 protein essential for cell cycle control at the G_1/S transition. *DBC2* also down-regulates CCNE, which is downstream from CCND1. Certain oncogenes involved in breast cancer development such as *ras*/c-*erb-B2* depend entirely on CCND1 to connect to the cell cycle machinery and some do not. If *DBC2* demonstrates antitumor activity via CCND1, it is conceivable that cancer cells will display different sensitivities against *DBC2*. In fact, *DBC2* expression causes growth arrest of a cancer cell line with CCND1 amplification, although it does not affect the growth of a cell line driven by an oncogene (c-*myc*). This finding warrants further questions: Are tumor cells using CCND1, such as cancers driven by *ras* and c-*erb-B2*

oncogenes, sensitive to *DBC2*? Can CCND1 or CCNE reactivation rescue the sensitive phenotype? How does *DBC2* suppress CCND1? We needed to answer these questions because they are directly related to the control of tumor growth by *DBC2*. To answer these questions, we constructed several mammalian expression vectors, including CCND1 with the constitutive promoter and CCNE with the CCND1 promoter. *DBC2*-sensitive tumor cells are now being examined to determine whether these constructs will allow growth even with *DBC2* activation.

DBC2-deficient Animal Model

M. Hamaguchi, N. Kobayashi, J.L. Meth

The development of "knockout (KO) mice" has created a system in which we can study gene functions at an organismal level. If inheritance of tumor susceptibility is proven by studying KO mice, it will provide decisive evidence that the targeted gene is a tumor suppressor. This has been a critical approach to the investigation of tumor suppressor candidates for sporadic cancers. Furthermore, studies in mouse models have provided information about the roles of targeted genes in cell cycle control, tumor suppression, DNA-damage response, regulation of apoptotic pathways, and preservation of genomic stability. Our attempts to produce *DBC2*-deficient mice have failed, and thus we are taking a conditional knockout strategy to circumvent the problem. We have successfully created mice carrying a "floxed-*DBC2*" allele that can produce functional *DBC2* and can be excised whenever we like. We are currently testing the responsiveness of the allele.

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RNA INTERFERENCE: MECHANISMS AND APPLICATIONS

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	S. Cheloufi			

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 continue to approach RNAi from three perspectives: working to decipher its underlying mechanism, seeking to uncover its biological functions in mammals, and building upon our knowledge of the pathway, to create tools that allow RNAi to be used to probe gene function in mammals and plants.

This year, our efforts have continued toward understanding how the RNAi machinery functions to suppress gene expression. In particular, we determined that miRNAs (miRNAs) may act by moving mRNA targets from a translating pool on ribosomes into a translationally inactive compartment known as the processing body or P-body. P-bodies are intriguing cytoplasmic structures that may act as sites of RNA storage and degradation, consistent with their role in miRNA-mediated silencing. However, these bodies may also be analogs of other RNA storage granules that are important for determining cell type and tissue identity, particularly RNP (ribonucleoprotein) granules such as P-granules or polar granules that specify the germ line during early development. Interestingly, miRNAs also direct degradative enzymes to cellular mRNAs, including the decapping complex and the major cellular deadenylases. These findings raise the possibility either that repression could occur through several independent mechanisms or that all of these factors lie in a coordinated pathway for negating mRNA function. Efforts not only to understand how miRNAs work, but also to decipher their biological roles led to the identification of the first oncogenic miRNA. We are now trying to understand how this miRNA functions in tumorigenesis through the development of new biochemical methods to identify miRNA targets.

In an effort to understand the biological roles of the RNAi machinery more broadly, we have begun to characterize its potential targets by large-scale sequencing of small RNAs. In particular, we have focused on the RISCs (RNA-induced silencing complexes) of *Arabidopsis*, both the miRNA RISC and the RISC involved in chromatin modification. Similar approaches in mammals have led to the discovery of a new class of small RNAs that appear exclusively in the germ line. These associate with a separate subfamily of Argonaute proteins, the Piwi proteins, and may have roles in meiosis. Overall, these efforts have continued to expand the biological range of small RNAs to previously unsuspected cellular processes.

Building on our increasingly deep understanding of the mechanistic basis and biological functions of RNAi, we continue to move toward the creation of genome-wide libraries of RNAi-inducing constructs to enable genetic studies in mammalian cells and in animals. Last year, we reported a nearly complete collection of human artificial miRNAs, targeting more than 30,000 genes. A similar mouse library has also been created. In collaboration with Scott Lowe and Steve Elledge here at CSHL, we showed that these tools can be used to effectively suppress gene expression in animals, even when only a single copy of the ectopic locus is present. We are now using these tools to search for genes that are essential for the growth and survival of cancer cells or that modify the sensitivity of cancer cells to therapeutic compounds.

During the past year, we were joined by three new graduate students: Angelique Girard from the Watson School, and Ingrid Ibarra and Xingyue He from the State University of New York. We were also joined by three new postdoctoral fellows: Katalin Fejes-Toth, Julius Brennecke, and Alexei Aravin, all coming from The Rockefeller University. Michelle Carmell and Ahmet Denli received their Ph.D. degrees, and Ahmet

left to take a postdoctoral position at the Salk Institute. We also said farewell to Mike Golding, a postdoctoral fellow, who left to continue his work at the University of Western Ontario.

Highlights of the progress in the laboratory during the past year are described in more detail below.

A New Class of Small RNAs in the Male Germ Line

A. Aravin, M. Carmell, A. Girard, E. Murchison

Small RNAs associate with Argonaute proteins and serve as sequence-specific guides to regulate mRNA stability, protein synthesis, chromatin organization, and genome structure. In animals, Argonautes segregate into two subfamilies. The Argonaute subfamily acts in RNAi and in miRNA-mediated gene regulation using 21–22-nucleotide RNAs as guides. The Piwi subfamily is involved in germ-line-specific events such as germ-line stem cell maintenance and meiosis.

We have recently shown that Miwi, a murine PIWI protein, binds a previously uncharacterized class of about 29–30-nucleotide RNAs that are highly abundant in testes. We named these piRNAs (PIWI-interacting RNAs). piRNAs show distinctive localization patterns in the genome, being predominantly grouped into 20–90-kB clusters, wherein long stretches of small RNAs are derived from only one strand.

We are now working to understand the biogenesis and function of the piRNAs. Dicer is an RNase III enzyme required for biogenesis of small RNAs entering the RNAi pathway. Although many features of piRNAs are indicative of RNase-III-mediated biogenesis, their size, distribution, and strand asymmetry are unusual for Dicer. The generation of mice with Dicer specifically ablated in testes will allow us to test the involvement of this enzyme in piRNA biogenesis. In addition, the identification and characterization of piRNA-precursor transcripts will permit us to probe the requirements for piRNA biogenesis in vitro.

Importantly, the function of this new class of small RNAs must still be resolved, the abundance of piRNAs in germ-line cells and the male sterility of Miwi mutants suggest a role in gametogenesis. For example, piRNAs could act in the homology search that precedes homologous chromosome pairing during meiosis. They could also mark meiotic recombination hot spots. Alternatively, their function could be more similar to that of known classes of small RNAs. They could work in establishing the chromatin structure or in regulating synthesis of spermatogenesis-associated proteins. We are planning to use a combination of mouse genetics and biochemical analyses to answer these questions.

The Role of P-bodies in miRNA-mediated Silencing

J. Liu

Small RNAs, including small interfering RNAs (siRNAs) and miRNAs, can silence target genes through several different effector mechanisms. In animals, siRNA-directed mRNA cleavage is carried out by the Argonaute2/slicer effector complex. However, the majority of miRNAs regulate gene expression through an RNAi machinery without inducing cleavage due to imperfect complementarity. The mechanisms by which miRNAs repress protein synthesis remain elusive. Our recent studies, in collaboration with Roy Parker, University of Arizona, demonstrate that Argonaute proteins, the signature components of RNAi effector complex, RISC, localize to specific cytoplasmic foci known as the mammalian P-bodies. The localization requires small RNA binding. More importantly, we demonstrate that the target mRNAs of miRNAs localize in P-bodies in a small-RNA-dependent manner. Disruption of the P-bodies impairs both siRNA- and miRNA-mediated silencing. Localization of RISC in P-bodies is crucial for its function. We propose that P-bodies serve as the cytoplasmic compartment for RISC effector complexes to carry out RNAi posttranscriptional gene silencing (PTGS). P-bodies contain untranslated mRNAs and serve as sites of mRNA degradation. The target mRNAs of miRNAs can therefore either be sequestered away from the translational machinery for repression or be degraded through a slicer-independent pathway. Further studies are required to dissect the details of the repression mechanisms.

Mechanisms of RNAi-mediated Silencing in Mammals

F. Rivas

Although cell biological studies have proven to be very informative, the precise biochemical mechanisms

by which miRNAs repress their targets remain a fundamental mystery. To illuminate this process, we have undertaken a proteomic approach to RISC in mammalian cells. To investigate mechanisms of RISC-mediated repression, we have searched for Argonaute-interacting proteins by MudPIT (*mul*ti*di*mensional *p*rotein *i*dentification *t*echnology) analysis of immunoaffinity-purified Ago1 and Ago2 complexes. These analyses have been possible by a collaboration with Dr. James Wohlschlegel and Dr. John Yates at The Scripps Research Institute. This approach was validated by the recovery of previously identified Argonaute-binding proteins including HSP90, Dicer, TRBP, and DCP1 in both Ago1 and Ago2 complexes. We also repeatedly identified a known component of processing bodies (P-bodies), the protein GW182. These observations were verified by immunoblotting GW182 immunoprecipitates for *myc*-tagged Ago1 and Ago2 and were shown to be RNase-A-insensitive. Our lab has gone on to show that knockdown of GW182 impairs the silencing of a miRNA reporter and that mutations in Ago which impair its ability to localize to P-bodies prevent miRNA-mediated repression even when Ago is tethered to its target in an siRNA-independent manner. Interestingly, these mutant Ago proteins were still able to bind GW182, suggesting that repression correlates with localization to the P-bodies as opposed to the ability to bind this integral P-body component. Our analysis of Ago MudPIT candidates has also repeatedly identified components of the CCR4-NOT (CNOT) complex. CCR4 is the most prominent deadenlylase activity in mammalian cells. Our findings are particularly provocative at this time since miRNA targets in zebra fish and in mammalian cells have recently been shown to undergo deadenylation. I have shown that CCR4 coimmunoprecipitates with Ago, as well as colocalize to the P-body with other members of the CNOT complex such as CNOT7 and CNOT10 and that knockdown of these proteins impacts miRNA-mediated repression. We are now investigating the biochemical mechanism that connects Ago to mRNA deadenlyation. We have also extended our MudPIT analyses to both nuclear and cytoplasmic Ago-interacting proteins, as well as asked whether these interactions were RNA-mediated or enhanced upon the triggering of the RNAi pathway by transfection of an siRNA. Remarkably, many novel Ago-interacting proteins identified by this approach are orthologs of proteins that have been previously ascribed a role in RNAi by genetic screens in *C. elegans*. In addition, some interactions reveal potentially novel roles for Argonaute proteins in the cell and are areas of current investigation in the lab.

miRNAs as Human Oncogenes

L. He

I am interested in looking at the role of miRNA in tumorigeneisis. To date, more than 200 miRNAs have been described in humans. Although there was circumstantial evidence suggesting that these regulatory, noncoding RNAs might have a role in tumor development, it remained largely obscure whether a miRNA could actually function as an oncogene. A comparison of B-cell lymphoma samples and cell lines to normal tissues reveals that substantially increased levels of the mature miRNAs from the *miR17-92* locus are often found in these cancers. Notably, the gene encoding this miRNA polycistron is present in a region of DNA amplification found in human B-cell lymphomas. Enforced expression of the *miR17-92* cluster cooperates with c-*myc*, accelerating tumor development in a mouse B-cell lymphoma model. Tumors derived from hematopoietic stem cells (HSCs) expressing *mir17-92* and c-*myc* are distinguished by an absence of the apoptosis otherwise prevalent in c-*myc*-induced lymphomas. Our studies indicate that noncoding RNAs, specifically miRNAs, can modulate tumor formation and implicate the *miR17-92* cluster as a potential human oncogene.

The Non-Agocentric View of RNAi

M. Dus

Much of the research in the field has centered on unraveling the biochemical details of target mRNA cleavage; however, very little is known about the in vivo functions of RNAi. The principal focus of my research stresses the importance of placing the RNAi pathway in a cellular contest in order to understand its physiological functions. Indeed, although RNAi is an extremely useful and profitable technique, it is first and foremost a biological process, a biological process of which we know very little. How do Argonaute proteins find the small RNAs? How does the microprocessor complex find the primary miRNA transcripts among the myriad of nuclear RNAs? How are pre-miRNAs transported into the cytoplasm? Do other mRNA processing complexes interact with the RISC

and, if so, what are the scopes and the defined mechanisms of these interactions? These and others are questions that I (and others in the lab) am trying to address with different approaches, from biochemistry, to genetics, to cell biology.

Biochemical Approaches to Identifying Small RNA Targets

F. Karginov

My research focuses on the function and mechanism of RNA silencing from a biochemical perspective. In one area of interest, I am identifying the function of bacterial Argonaute proteins. Although bacteria lack the full set of canonical eukaryotic RNAi components, several bacterial species have a homolog of Argonaute, the principal effector protein in RNA silencing. Although the *Aquatex aeolicus* Argonaute has been shown to be active in slicing, other bacterial Agos lack the necessary catalytic residues, presumably participating in other functions. I believe that elucidation of bacterial cellular processes that involve Argonautes will provide a broader understanding of their potential functions in eukaryotes and may explain their ancestral roles and evolution.

I am also interested in experimental identification of miRNA targets. To date, computational methods have identified hundreds of putative mRNA targets for given miRNAs, and microarray hybridization experiments have yielded information on changes in mRNA levels upon introduction or depletion of particular miRNAs. I am working on directly identifying miRNA targets by immunoprecipitation of RISCs. In this approach, secondary effects seen in microarray experiments can be avoided, and we can begin to illuminate the extensive networks of gene regulation by miRNAs with more precision. Additionally, a large set of experimentally verified mRNA-miRNA pairs will lead to substantial improvement of computational algorithms for more target prediction.

Genetic Approaches to New Therapeutic Targets for Cancer

K. Chang, J. Silva, D. Siolas, S. Boettcher

The application of RNAi has transformed the way we approach mammalian cell genetics. During the past year we have made significant progress in several areas that have enhanced the use of short hairpin RNAs (shRNAs) as a tool for genetic screens. Our second-generation shRNA retroviral library now covers 32,202 genes (86,128 clones) for human, 30,629 genes (76,896 clones) for mouse, and 15,173 genes (31,130 clones) for rat genomes. For screening using primary or quiescent cultures, we have begun to construct a lentiviral human shRNA library (12,099 genes; 15,471 clones). These shRNA can be delivered into cells, both in vitro and in vivo, using our optimized viral expression vectors. More importantly, with these tools, we have successfully demonstrated the feasibility of performing genetic screens in mouse and human cells through our pilot efforts. A screen to search for potential tumor suppressors in human breast cancer cells was undertaken by isolating cells that could survive detachment-induced apoptosis ("anoikis"). As a validation of our approach, we were able to identify previously known tumor suppressors (PTEN and Rb). Currently, verification is under way to confirm some of the novel targets. Using the "synthetic-lethal" genetic approach, we can now perform drug-induced synthetic-lethal screens by using cancer drugs that are currently in clinical trials. This is important as cancer cells can often develop growth advantage to overcome the toxic effects of chemotherapeutics. To this end, we have carried out a screen to identify regulators of rapamycin (an inhibitor of mTOR) sensitivity in prostate cancer cells and have identified genes that were expected to modulate mTOR activity and other targets which, upon validation, could become candidates for combination therapy against prostate cancer. Bortezomib (Velcade) is the first targeted therapeutic to the proteasome approved by the FDA for treatment against multiple myeloma and is currently in phase II clinical trials for breast and lung cancers. Our goal is to identify genes that mediate resistance against Velcade that could serve as potential drug targets. Screens to investigate genes that modify sensitivity to other leading targeted therapeutics, such as Tarceva (EGFR inhibitor), are also being carried out. It will be interesting to compare results across these studies to identify generic effects of drug resistance.

RNAi technology is a powerful tool that could potentially be used to study and treat other human diseases through its application to mammalian cell genetics.

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SPORADIC HUMAN GENETIC DISEASE

E. Hatchwell Y. Hanada D. Tegay
J. Roohi Y. Wang

We continue to apply modern/novel methodologies to the analysis of sporadic human genetic disorders. Approximately 2–3% of all newborns have a disorder that is at least partially genetic in origin; of these, approximately 50% are sporadic and, by definition, not amenable to standard approaches, including linkage analysis.

Historically, whole-genome analysis was based on examination of chromosomes using light microscopy. Although highly successful, this approach is limited by a maximum resolution of 10 Mb. It is now known that many disorders are associated with genomic copy-number variation of much smaller segments (e.g., Williams-Beuren Syndrome [WBS], which is associated with heterozygous deletions of 1.5 Mb at 7q11.23).

Array-based comparative genomic hybridization (aCGH) for the genome-wide detection of copy-number fluctuations is now possible using a variety of formats, including oligo and large-clone (based on bacterial artificial chromosomes [BACs]) DNA micro-arrays.

We have generated BAC microarrays during the last four years, at increasing density, and our current arrays contain a complete tile of the human genome (19,000 BACs arrayed in duplicate on a single slide), with an average expected resolution of 100 kb. These were designed in collaboration with James Gergel and were generated by collaborators at Roswell Park Cancer Institute ([RPCI] Dr. Norma Nowak and colleagues). We continue to focus on specific areas:

- Autistic spectrum disorder (ASD)
- Congenital heart disease (CHD)
- Complex phenotypes and de novo, apparently, balanced translocations
- Development of a normal variation database, for rational interpretation of copy number changes detected in symptomatic patients
- Development of methods to generate haploidy from diploidy, in order to more effectively analyze heterozygous changes
- Genome-wide array-based analysis of DNA methylation for both inter- and intragenomic analysis

Autism Spectrum Disorder: Genetics

J. Roohi

We are collaborating with workers at the Cody Center, Stony Brook, in a multidisciplinary study of a cohort of patients diagnosed with ASD. In addition to detailed diagnostic interviews/questionnaires by experts in autism, all individuals undergo examination by a medical geneticist (Dr. David Tegay) in order to search for dysmorphic features. All patients undergo standard chromosome analysis and testing for FRAXA (Fragile-X type A). To date, more than 80 patients have been assessed. Chromosomal analysis was reported to be normal in all cases, except one patient who has a de novo, apparently balanced, translocation, which we intend to study in great detail. What has become very clear is how clinically heterogeneous this disorder is; fully 50% of patients are dysmorphic or have physical stigmata reminiscent of genetic syndromes. To some extent, this is not altogether surprising, given the number of distinct syndromes known to result in autistic features (e.g., FRAXA, Angelman syndrome, and Rett syndrome). It is therefore clear that ASD should be viewed as a "catch-all" diagnosis for a large group of heterogeneous disorders whose common endpoint is the autistic phenotype. In a sense, the ASD label is likely to disappear in the future with improved phenotypic dissection, as has occurred in other complex disorders whose etiologies are beginning to be understood (e.g., mental retardation, now considered too general a designation to be useful). It is also important to realize that the application of any genomic technology to unravel ASD or any complex phenotype, in the absence of a parallel clinical/phenotypic analysis, is unlikely to be informative.

In addition to our medical genetics examinations, a selected group of patients undergo detailed imaging analysis, in collaboration with Dr. Benveniste and colleagues at Brookhaven National Laboratory.

We have been applying aCGH using tiling-path BAC arrays to our cohort of ASD patients and have detected many genomic copy-number abnormalities. The challenge has been to (1) validate these changes

and (2) determine whether any validated changes represent population variants or are specific to ASD.

Our validation relies mainly on "old-fashioned" yet robust methods such as fluorescence in situ hybridization (FISH), although we are also utilizing a variety of molecular approaches, including quantitative polymerase chain reaction (PCR), multiplex ligation-dependent probe amplification (MLPA), and short tandem repeat (STR) analyses.

We have generated a database of all our aCGH results, which has enabled us to determine with high likelihood which changes are likely to be significant in any given disorder. Our database currently contains data on more than 320 individuals; this number is likely to rise to close to 500 by the summer. The data have been generated in both our group and Dr. Norma Nowak's group at RPCI.

We are currently interested in a specific region of the genome that appears to be normal in all non-ASD individuals we have looked at so far. We are analyzing candidate genes for a variety of mutational types and are fully confident that we have found a locus that will turn out to be highly significant in a subset of ASD (perhaps 5%).

ASD: Environment

This work was performed in collaboration with M. Herbert, Massachusetts General Hospital, Harvard Medical School.

We have embarked on a study of the possible role of environmentally responsive genes in ASD etiology. Although there is much debate, there does exist some evidence for a true increase in the rates of ASD during the last 20 years. If real, these increases, which have occurred in populations whose demography/ethnicity has changed little, require explanation in terms of environmental changes. We have used bioinformatic approaches to analyze potential overlaps between genes known to be implicated in responses to the environment (http://www.niehs.nih.gov/envgenom/egp6.htm) and ASD linkage regions and have selected a small subset for further study.

Congenital Heart Disease

Y. Wang

We have a long-standing interest in the genetics of CHD; 8/1000 newborns have CHD. Of these, only 3% are Mendelian, and the majority of the remaining 97% are of unknown etiology, with one major exception. Velocardiofacial syndrome (VCFS) is a significant cause of CHD, explaining about 20% of all conotruncal defects (and probably 10% of all CHD). VCFS is known to be caused by submicroscopic deletions at chromosome 22q11 (3 Mb in 90% of patients, 1.5 Mb in about 10%). It therefore seems reasonable that other genomic copy-number alterations will be found in patients with CHD who are chromosomally normal and negative for 22q11 deletions.

We have funding from two organizations for this work: (1) Harrison's Heart Foundation, a private local charity committed exclusively to CHD research, and (2) the Gerber Foundation, which funds research more generally in the pediatric arena. We also have active collaborations with two major centers of excellence, from which we obtain DNA samples on well-characterized individuals with CHD: (1) Dr. Piers Daubeney at The Brompton Hospital, London and (2) Dr. Steve Webber at The Children's Hospital, Pittsburgh.

To date, we have obtained samples from 150 patients with CHD and have thus far analyzed 50 individuals. By comparison with non-CHD individuals in our database, we have identified at least one region that appears to be of interest.

Complex Phenotypes and De Novo Translocations

Y. Wang, D. Tegay

The methods we have developed for studying ASD and CHD are applicable to a wide range of complex disorders. We collaborate actively with a large number of clinicians, including Dr. Helga Toriello, a distinguished dysmorphologist, and Dr. David Tegay, Head of Medical Genetics at Stony Brook, currently a visiting scientist in our group.

De novo translocations may provide important clues to the etiology of certain phenotypes. In one such example, which we have studied in depth, we have cloned a novel gene that is interrupted by a de novo t(2; 22) translocation in a patient with Joubert syndrome. In a second individual, who has Toriello-Carey Syndrome, we have isolated a candidate gene and have amassed a large collection of individuals with this very rare syndrome, in whom mutation analysis is under way.

In addition to these projects, we have recently uncovered *relevant* copy-number changes in:

- A patient with atypical Williams Syndrome. This patient serves to better define genotype/phenotype correlations with regard to the cognitive features in this condition.
- A patient with Smith Magenis Syndrome (SMS) in whom mutations of RAI1 have been excluded. We collaborate with Dr. Sarah Elsea (Virginia Commonwealth University), who has furnished us with 24 patients with SMS unrelated to RAI1 mutations.

Development of Methods to Generate Haploidy from Diploidy

Y. Hanada

Once copy-number fluctuations are validated, it is often of interest to study these in greater detail to determine precise genomic boundaries and to understand possible genomic structural predispositions to rearrangement. Liquid DNA is diploid (unless isolated from single sperm), and it is problematic to separate the two alleles for further study. A traditional approach to separating chromosomal homologs is the generation of somatic cell hybrids. This method, although robust, is extremely time consuming and labor intensive.

We are experimenting with the separation of chromosome homologs by universal amplification of dilutions of chromosome suspensions generated from patients of interest. Chromosomes are diluted to final concentrations of approximately 23 chromosomes per well in a 96-well plate, and the material is amplified using multiple displacement amplification with the highly processive enzyme phi29. PCR analysis of the amplified material is then used to determine whether the two homologs have been separated in different wells of the same plate. Our results to date are encouraging, both in terms homolog separation and in our ability to avoid contamination with exogenous DNA.

Genome-wide Array-based Analysis of DNA Methylation

E. Hatchwell

Intergenomic Methylation Comparison: MZ Twins. The underlying causes of phenotypic variation in human genetic disorders are still largely not understood. Although "genetic background" is often invoked to explain variation in nonidentical individuals, this appears not to be relevant to monozygotic (MZ) twins (although somatic mutations cannot be excluded). One possible source of phenotypic variation in MZ twins is the epigenome—the state of the genome unrelated to DNA sequence variation. Preliminary evidence suggests that MZ twins may vary greatly in their methylation. Such studies have, however, been limited to the examination of specific genes.

We have designed and optimized a genome-wide array, based on the generation of oligonucleotides, in collaboration with NimbleGen. Our arrays are based on the use of small *Hpa*II fragments from the human genome. *Hpa*II cleaves at CCGG, only when the internal C is not methylated. By recovering small *Hpa*II fragments from the genome, it is possible to obtain a genome-wide picture of DNA methylation. We have studied five sets of MZ twins, both normal and discordant for reading ability, a collaboration with Professor Ian Craig, Institute of Psychiatry, London. Preliminary analysis reveals dramatic differences at some loci and general differences genome-wide between concordant and discordant MZ twins.

This work was performed in collaboration with J. Greally, Albert Einstein College of Medicine.

Intragenomic Methylation Analysis. We have contributed to the development of a novel methodology known as the HELP assay (*Hpa*II tiny fraction [HTF] enrichment by ligation-mediated PCR). In this method, a comparison is carried out between small *Msp*I and small *Hpa*II fragments from the same genome. This allows for an intragenomic methylation profile to be built up. The data have been extensively validated using pyrosequencing and has demonstrated dramatic tissue-specific differences in methylation.

THE ORIGIN AND VIABILITY OF CANCER CELLS

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

Our research focuses on two questions: How are normal cells converted into cancer cells? How can cancer cells be killed by manipulating the mechanisms that control their viability?

CELL FUSION AS A COFACTOR IN CARCINOGENESIS AND TUMOR PROGRESSION

Our interest in the first question was triggered by an accidental observation that cells in tissue culture fuse with each other. Studies of published reports on cell fusion revealed that it is a process that induces aneuploidy, a hallmark of cancer cells, and further increases cell diversity by combining, segregating, and rearranging epigenetic and other regulatory networks of the fusion partners. A century ago, the observation that cell fusion causes aneuploidy led to a hypothesis, which has been evolving, that accidental cell fusion can produce cancerous cells. If cell fusion can indeed lead to cancer, one question is how this fusion is induced.

In the body, fused cells come from two sources: physiological and aberrant cell fusion. Physiological cell fusion is a part of normal development that is required to produce the zygote and several types of multinuclear somatic cells. Importantly, with the exception of the zygote, cells produced by physiological cell fusion are terminally differentiated and do not proliferate. Aberrant cell fusion can be caused by a variety of agents, including viruses, chemicals, and fibers (e.g., asbestos) or by abnormal expression of cellular proteins.

Viruses are of particular interest as pathological fusogens because they are ubiquitous, diverse, amplifiable, can persist in cells inconspicuously, and have been implicated in carcinogenesis. Yet, cells fused by viruses are considered harmless because they die. We provided evidence that a primate virus uses both viral and exosomal proteins involved in cell fusion to produce transformed proliferating human cells. While normal cells indeed fail to proliferate after fusion, expression of an oncogene or a mutated tumor suppressor *p53* just in one of the fusion partners is sufficient to produce heterogeneous progeny. We also demonstrated that this virus can produce viable oncogenically transformed cells by fusing cells that are otherwise destined to die. Therefore, we proposed that viruses can contribute to carcinogenesis by fusing cells (Fig. 1).

In particular, our model proposes how a virus can contribute to carcinogenesis or tumor progression without becoming a part of the affected cells. Indeed, a current view is that viruses transform cells by introduction of oncogenes or by modifying normal gene expression through integration into the host genome. This concept implies that if a virus is an etiological agent of cancer, cancer cells must carry or have carried the viral genome. Our model implies that a virus may produce cancer cells without infecting them, a notion that may help to understand better the numerous correlative links between retroviruses and human cancers.

METABOLIC CHANGES AS A BASIS FOR KILLING CANCER CELLS SELECTIVELY

The second part of our research is based on the notion that understanding how cancer cells are generated may not provide a clue on how to kill them. Therefore, we are exploring differences between normal and cancer cells that allow for their selective or preferential killing of cancer cells. In a search for these differences, we have been focusing on apoptosis and, in particular, on its regulation by 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 are pro-apoptotic activities specific for transformed cells. If true, then

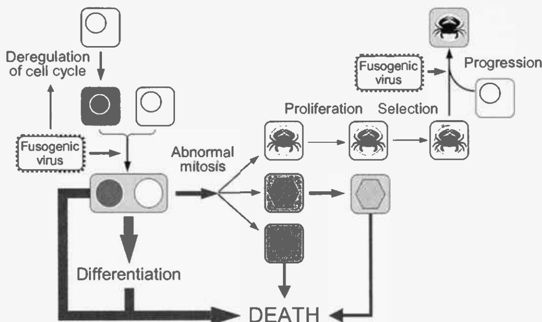

FIGURE 1 Cell fusion as a link between viruses and carcinogenesis. Illicit cell fusion induced by viruses has no consequences for carcinogenesis if resulting cells either die or do not proliferate. However, events that deregulate the cell cycle, which includes viral proteins such as E1A or E7, enable proliferation of fused cells. The majority of these cells die within a few cell divisions because of chromosomal aberrations associated with abnormal mitoses or other consequences of cell fusion. The few cells that survive have a deregulated cell cycle, lack normal response to this deregulation, are aneuploid, and have epigenetic regulation that is an unpredictable result of the merger between fusion partners. A combination of these properties in a minute fraction of the fused cells may be sufficient to make them cancerous. Considering that a single cell can give rise to a cancer, the frequency with which cell fusion produces such cells does not need to be high to have pathological consequences. A cancer cell may fuse to another cell, thus increasing diversity of a tumor.

understanding how the apoptotic machinery is regulated by oncogenes may help to develop ways to kill cancer cells selectively.

A prevailing approach for activating caspases in cancer cells is cell damage that induces activation of the apoptotic machinery. For largely historical reasons, this damage usually involves DNA, a molecule that is identical chemically in normal and transformed cells, and modifications of which often cause mutations, some of which can lead to cancer. An evolving alternative approach to selective killing of cancer cells is based on exploiting metabolic differences between normal and cancerous cells.

Our way of developing this approach was to investigate how expression of E1A or Myc affects metabolism of human cells and, in particular, how these oncogenes change dependence of the cells on major nutrients. We found that, in contrast to previous studies, expression of Myc does not affect sensitivity of the cells to glucose withdrawal, as both normal cells and cells that express Myc die without this nutrient, and cell death has no signs of apoptosis. However, we found that depletion of glutamine induces apoptosis in cells that express Myc but does not affect viability of normal cells. Previously, glutamine depletion and glutamine analogs that interfere with metabolism of this amino acid were exceptionally efficient in mouse models of cancer and were used in the clinic, but they were abandoned because of their toxicity. We thought that by understanding how glutamine deficiency causes cell death in transformed cells, we would be able to suggest how to retain the therapeutic benefits of the previous treatments but avoid their toxicity.

During this year, we tested whether interfering with multiple pathways of glutamine metabolism would mimic the effect of glutamine depletion on cell viability. We found that interfering with none of these pathways could kill cells as efficiently as did glutamine depletion. In September of this year, Dr. Maria Yuneva, who was working on this project moved to Dr. Michael Bishop's lab at University of California at San Francisco (UCSF) where she continues this line of investigation in collaboration with us.

Overall, this part of our studies continued to use simple experimental systems in order to understand how cancer cells can be killed selectively.

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REGULATION OF APOPTOSIS AND SENESCENCE BY CANCER GENES

S. Lowe A. Bric, D. Burgess, E. Cepero, C. Chang, A. Chicas, R. Dickins, K. Diggins, M. Hemann, Y. Hippo, V. Krizhanovsky, M. McCurrach, M. Narita, M. Narita, S. Nuñez, P. Premsrirut, C. Rosenthal, M. Spector, H. Wendel, W. Xue, M. Yang, L. Zender, Z. Zhao, J. Zilfou, J. Zuber

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 have increasingly relied on new types of animal models and gene manipulation technologies to study tumor development and cancer therapy in vivo.

Control of Apoptosis by Oncogenes Tumor Suppressor Genes

A. Bric, D. Burgess, E. Cepero, M. Hemann, M. McCurrach, M. Narita, W. Xue, L. Zender, J. Zilfou [in collaboration with former laboratory member A. Samuelson]

Normal cells possess intrinsic tumor suppressor mechanisms that limit the consequences of aberrant proliferation. For example, deregulated expression of the 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 through both p53-dependent and independent pathways and potently 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 transform normal cells in vitro and promote tumorigenesis in vivo. We are currently interested in identifying additional components of these programs and understanding how they function in a "tumor suppressor network."

Historically, we have used the E1A oncoprotein to study how proliferation is coupled to apoptosis. Using a structure-function approach, we recently showed that E1A induces ARF and p53 and promotes apoptosis in normal fibroblasts by physically associating with Rb and a p400/TRRAP complex, and that its interaction with p300 is largely dispensable for these effects (Samuelson et al. 2005). We further showed that E1A increases p400 expression and, conversely, that suppression of p400 using stable RNA interference reduces the levels of ARF, p53, and apoptosis in E1A expressing cells. Therefore, although E1A inactivates Rb, it requires p400 to efficiently promote cell death. More recently, we collaborated with William Tansey and Gregory Hannon to explore how E1A binding to p400 contributes to E1A's apoptotic potential and transforming capabilities.

Like E1A, the cellular c-Myc oncoprotein drives proliferation and cell death. In Burkitt's lymphoma, this deregulation occurs via reciprocal translocations that juxtapose c-*myc* with an immunoglobulin (Ig) promoter, leading to gross overexpression of c-*myc* mRNA in the B-cell lineage. In addition, point mutations are often found in the translocated *myc* alleles, clustering in a conserved region known as Myc box I. Although some mutations can increase Myc stability and transforming activity in vitro, their impact on the pathogenesis of Burkitt's lymphoma is unclear. In fact, translocated c-*myc* genes are subject to hypermutation in vivo that can also alter noncoding sequences, raising the possibility that these mutations are a consequence and not a cause of tumor development.

This year, we showed that two common mutant *myc* alleles derived from human Burkitt's lymphomas uncouple proliferation from apoptosis and, as a result, are substantially more effective than wild-type *myc* at promoting B-cell lymphomagenesis in mice (Fig. 1)

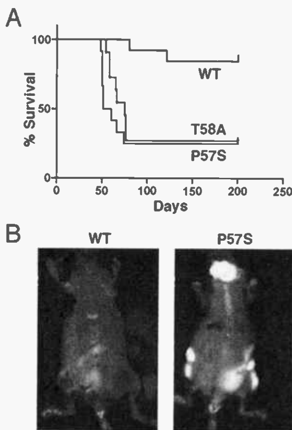

FIGURE 1 Tumor-derived *myc* mutants are highly oncogenic in vivo. Mice were reconstituted with hematopoietic stem cells expressing wild-type *myc* or two tumor-derived *myc* mutants (P57S and T58A) together with green fluorescent protein. Animals were monitored for tumorigenesis using lymph node palpation (*A*) and whole body fluorescence imaging (*B*) (Hemann et al. 2005).

(Hemann et al. 2005). Mutant Myc proteins retain their ability to stimulate proliferation and activate p53, yet are defective at promoting apoptosis due to a failure to induce the BH3-only protein Bim and effectively inhibit Bcl-2. Disruption of apoptosis through enforced expression of Bcl-2, or loss of either *Bim* or *p53*, enables wild-type myc to produce lymphomas as efficiently as mutant myc. These data reveal how parallel apoptotic pathways act in concert to suppress Myc-induced transformation and how mutant Myc proteins, by selectively disabling a p53-independent pathway, allow tumor cells to evade p53 action during lymphomagenesis.

Control of Cellular Senescence

D. Burgess, C. Chang, A. Chicas, V. Krizhanovsky, M. Narita, M. Narita, S. Nuñez

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 suboptimal cell culture conditions. These observations have led us to propose that senescence acts in parallel to apoptosis as a cellular response to stress and acts in a similar way to suppress tumorigenesis and mediate responses to chemotherapy (for review, see Narita et al. 2005). Indeed, on the basis of this analogy, much of our work on senescence is guided by our past experience on apoptosis.

We continue to identify and characterize new factors that modulate senescence, with a particular emphasis on the senescence machinery, i.e., molecules that contribute to the irreversibility of the arrest. We recently reported that some senescent human fibroblasts accumulate a novel type of chromatin structure (named senescence-associated heterochromatic foci or SAHF) that is dependent on the Rb tumor suppressor and is associated with the stable silencing of E2F target genes. To gain insight into the chromatin changes linked with senescence, we have begun a proteomics analysis to identify molecules that selectively associate with chromatin in senescent cells. These studies have identified the high-mobility-group A (HMGA) proteins as proteins that accumulate on chromatin in senescent cells, where they localize with SAHFs. These results are paradoxical, as HMGA proteins have previously been linked to oncogenic transformation. Unraveling the role of HMGA proteins in senescence and their paradoxical role in cancer is one of our current challenges.

Modulation of Gene Expression In Vivo Using RNA Interference

R. Dickins, M. Hemann, P. Premsrirut, W. Xue, L. Zender, J. Zilfou [in collaboration with G. Hannon and L. He, Cold Spring Harbor Laboratory]

RNA interference (RNAi) is a powerful method for suppressing gene expression in mammalian cells. Stable knockdown of gene expression can be achieved by continuous expression of synthetic short hairpin RNAs (shRNAs), typically from RNA polymerase III promoters. However, primary microRNA transcripts (pri-miRNAs), which are endogenous triggers of RNAi, are normally synthesized by RNA polymerase II (pol II). In collaboration with Gregory Hannon, we showed that

pol II promoters expressing rationally designed pri-miRNA-based shRNAs (shRNA^{mir}) produce potent, stable, and regulatable gene knockdown in cultured cells and in animals, even when present at a single copy in the genome (Dickins et al. 2005). Most notably, by tightly regulating p53 knockdown using tetracycline-based systems, we showed that cultured mouse fibroblasts can be switched between proliferative and senescent states and that tumors induced by p53 suppression and cooperating oncogenes regress upon p53 reexpression. In practice, this shRNA^{mir} vector system is remarkably similar to cDNA overexpression systems and should be a powerful tool for studying gene function in cells and animals. We are taking advantage of these tools to explore various aspects of tumor suppressor gene networks in vitro and in vivo (see, e.g., Ramiero et al., in press; Zender et al., in press].

In parallel to these efforts, we have also collaborated with Dr. Hannon to study the oncogenic potential of endogenous microRNAs (miRNAs). To date, more than 200 miRNAs have been described in humans; however, the precise function of these regulatory, noncoding RNAs remains largely obscure. A comparison of B-cell lymphoma samples and cell lines to normal tissues reveals that substantially increased levels of the mature miRNAs from the miR17-92 locus are often found in these cancers. Notably, the gene encoding this miRNA polycistron is present in a region of DNA amplification found in human B-cell lymphomas. Enforced expression of the miR17-92 cluster cooperates with c-*myc*, accelerating tumor development in a mouse B-cell lymphoma model (He et al. 2005). Tumors derived from hematopoietic stem cells expressing *mir17-92* and c-*myc* are distinguished by an absence of the apoptosis that is otherwise prevalent in c-*myc*-induced lymphomas. These studies indicate that noncoding RNAs, specifically miRNAs, can modulate tumor formation and implicate the miR17-92 cluster as a potential human oncogene. We continue to work with the Hannon group to characterize the oncogenic properties of mIR17-92.

A New Mouse Model of Hepatocellular Carcinoma

Y. Hippo, M. Spector, W. Xue, L. Zender [in collaboration with R. Lucito, D. Mu, S. Powers, and M. Wigler, Cold Spring Harbor Laboratory; C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center; and P. Flemming, Hanover Medical School]

Hepatocellular carcinoma (HCC) represents the fifth most frequent neoplasm worldwide. However, due to a lack of treatment options, it constitutes the third leading cause of cancer death (>500,000 deaths per year). The only curative treatment options for HCC are surgical resection or liver transplantation. Unfortunately, at the time of diagnosis, the majority of patients have an advanced tumor and are not candidates for surgical therapy. Systemic chemotherapeutic treatment is ineffective against HCC, and so there is a great need for new therapies to treat this disease.

Our understanding of the molecular genetics and biology of liver cancer lags behind that of other major cancers, and these deficiencies represent a major obstacle to the development of new therapies. To better study this disease, we generated a new mouse model of hepatocellular carcinoma that is based on the ex vivo manipulation of liver progenitor cells, followed by the seeding of these cells into normal recipients. These methods allowed us to rapidly produce in situ liver cancers of defined genetic origin. After retroviral gene transfer of oncogenes or shRNAs targeting tumor suppressor genes, genetically altered liver progenitor cells are seeded into the liver of otherwise normal recipient mice. We show that histopathology of the engineered liver carcinomas reveals features of the human disease. Furthermore, representational oligonucleotide microarray analysis (ROMA) of murine liver tumors initiated by two defined genetic hits revealed spontaneously acquired genetic alterations that are characteristic for human hepatocellular carcinoma. This model provides a powerful platform for applications like cancer gene discovery and high-throughput preclinical drug testing. Working with collaborators at CSHL, we are using this to interrogate the genetics and biology of liver cancer, with the ultimate goal of using this model to validate or test new therapies.

Molecular Genetics of Drug Sensitivity and Resistance

D. Burgess, M. Hemann, Y. Hippo, M. McCurrach, C. Rosenthal, H. Wendel, M. Yang, L. Zender, J. Zilfou, J. Zuber [in collaboration with C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center; S. Kogan, University of California, San Francisco]

A major goal of our research is to understand the biological and molecular basis of drug sensitivity and resistance in tumors. Conventional approaches to identify factors that dictate treatment sensitivity often 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. One 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 to conventional chemotherapy in vivo.

More recently, insights into the molecular basis of cancer have enabled the development of more rational drugs that attack activities involved in the oncogenic process. These "targeted" therapeutics are often less toxic than conventional drugs and have remarkable activity against some cancers. Nevertheless, de novo or acquired resistance to these agents remains a clinical problem. In an effort to understand factors that influence resistance to targeted agents, we are studying that action of the drug Gleevec in a mouse model of myeloid leukemia. Gleevec is a small-molecule inhibitor of the Bcr-Abl oncoprotein (the cause of chronic myelogenous leukemia) and represents the paradigm of targeted therapy.

Using a mouse model of Bcr-Abl-induced leukemia, we demonstrated that p53 potentiates the action of Gleevec in vivo, such that mice harboring p53-deficient leukemias are more resistant to this agent. We believe that this reflects a new mode of resistance to targeted cancer therapy, and we will continue to explore the basis for this effect. We are also continuing a multi-institutional effort to use mouse models of acute myelogenous leukemia to identify molecular determinants of both conventional and targeted therapies, with the goal of translating this information to human patients. We are excited about the potential of the program, as it provides all of the elements necessary to validate the use of new mouse models as preclinical test systems and translate this information into clinical trials.

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GENOMIC MICROARRAY ANALYSIS OF CANCER

R. Lucito T. Auletta S. Chen O. Dovirak S. El-ftesi S. Khan K. Kuntz

Mutation of the genome is central to the development and progression of cancer. Mutations occur in the genome of 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 become subverted to allow the cell to become cancerous. We have developed a genomic microarray technique, 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 390,000 probes.

We have continued 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 gene 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 with clinical outcome. 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 those, 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, 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 lifespan after diagnosis is very short, the number of patients who 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. Currently, we are collaborating with Dr. Ralph Hruban of Johns Hopkins University, Dr. Daniel Von Hoff of the Arizona Cancer Center, and Dr. Vijay Yajnic of Massachusetts General Hospital, who will be providing pancreatic specimens and invaluable clinical information and expertise. To date, we have analyzed 66 samples composed of 33 primary tumors, 15 cell lines, and 18 xenografts. In addition, we have compiled published data for an additional 16 primary tumor samples into our data set to increase the information content. We have confirmed that many of the known mutated regions are altered in this set, including genes such as the INK4a-ARF locus, SMAD4, p53, and c-*myc* to name a few. In addition, there are other less characterized regions deleted (2p23, 3p, 4q23, 5p15, 10p15, and 12p) and amplified (1q42-43, 3q26, 6p21, 7p21, 14q21, 18q11, and 19q12) frequently in this cancer, and we are continuing to study these regions to identify possible gene candidate tumor suppressors and oncogenes.

Although we are working on several regions at this time, we will summarize one region amplified on

19q12 in specifics. A number of tumors and cell lines have this region amplified, but with two tumors that have the most informative amplicons, the epicenter or common region of mutation was delimited to approximately 2 million bases. This region contains 20 gene candidates. In the samples that have gene amplification, it is highly likely that the target gene is overexpressed at the RNA level, and the nontarget genes will not be overexpressed, although they may be overexpressed as a consequence of being gene-amplified. To decrease the number of viable gene candidates, gene expression analysis was performed on the cell lines and one of the primary tumors that have the amplicon. After this analysis was completed, the number of candidates was decreased to only two. Immunohistochemistry on one of the tumors that had gene amplification clearly demonstrated that one candidate was expressed highly in the normal as well as the tumor sample. The other candidate, a gene called p21-activated kinase or PAK4, was nearly absent in the normal but was found at extremely high levels in the tumor sample tissue. To determine if the encoded protein is active, we performed an in vitro kinase assay on extracts from cell lines that have the amplicon. In comparison to a cell line that did not have amplification of this gene, the cell lines had 10–15 more protein and 8- to 10-fold more kinase activity. Mutated or activated forms of PAK4 have been shown to increase tumorigenicity. We are presently sequencing the PAK4 gene in these samples to determine if the gene present encodes a constitutively active protein. However, another way of activating PAK4 is to activate RAS, an extremely common event in pancreatic cancer. Codon 12 (the most commonly mutated codon) of RAS was sequenced for these samples and four of five have an oncogenic form of the RAS protein. It is possible that PAK4 itself is activated in this fifth sample, and we will dermine this shortly. In the future, we plan to use short hairpin RNA (shRNA) constructs to knockdown the level of PAK4 in cells with the amplicon to determine the effect on tumorigenicity. We will also be moving this gene into an animal model for pancreatic cancer.

OVARIAN CANCER

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 SUNY Stony Brook 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.

At present, we have analyzed 75 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.

One such region we are further characterizing is a deletion on chromosome 5q14. This region is relatively small and contains only seven gene candidates. Since there were so few candidates in this region, we performed quantitative PCR (QPCR) on the mRNA of a large panel of cell lines and primary ovarian tumors. Presently, we have removed all candidates from analysis based on the expression data (similarly to what we have done for the pancreatic region discussed above), and have three candidates to further characterize. One gene in this region, *Centrin 3* (*CETN3*), is of particular interest. *CETN3* is a component of the centrosome and takes part in the cellular duplication process. In lower organisms, there is only one centrin, and experimental deletion of this gene resulted in centrosome amplification and aneuploidy, a common phenotype of cancer cells. We are presently studying whether the removal of this gene contributes to aneuploidy in cancer cells.

Methylation-specific Oligonucleotide Microarray Analysis (MOMA)

O. Dovirak, S. Chen, K. Kuntz, S. Khan

In addition to genetic mutation such as amplification and deletion, there are epigenetic mechanisms used to influence the transcriptional activity of a gene. One such mechanism 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 also 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-specific oligonucleotide microarray analysis (MOMA). Currently, we are utilizing this procedure 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 that do not involve genetic mutation.

We have taken advantage of several enzymes that discriminate methylated and unmethylated cytosines (MOMA). For example, a sample can be treated with two restriction enzymes, *Mcr*BC, which cleaves at (G/A)^{m}C ($N_{40-3000}$) (G/A)^{m}C, and *Msp*I, which cleaves at all sites of CCGG, in effect depleting the representation of fragments that have methylated CpGs. A second aliquot of the same sample is mock-digested for *Mcr*BC and digested with *Hpa*II, which is an isoschizomer of *Msp*I (recognizes the same sequence) but cleavage is blocked if the cytosine is methylated. The two differently processed samples are then compared.

We are continuing to develop this method. We have redesigned the array to represent known CpG islands of the genome, to make an array that is more specific for methylation detection and transcription control. We have tested the array with several samples and have demonstrated that it can discriminate when a CpG island is methylated or not methylated. We can also see, as others have reported, that when a cell becomes cancerous, there is a paradoxical decrease in global methylation and an increase in CpG island methylation. We have analyzed a cell line that has been reported to have the p16 CpG island hypermethylated. After analyzing this sample by MOMA, we can identify that the p16 promoter is more methylated than in normal samples. We plan to perfect this method so that it can be utilized to rapidly analyze the methylation state of the CpG islands in the genome. It is becoming clear that methylation silencing has an important role in cancer, and we intend to marry both techniques—ROMA and MOMA—as well as expression analysis to obtain a more complete picture of tumorigenesis. Both mutation and methylation have an effect on the level of gene transcription, a good example being the deletion of one copy of the INK/ARF locus and the hypermethylation of several of the CpG islands within this locus, specifically that in front of the *p16* gene. 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 whether any genetic or epigenetic factors correlate with clinical outcome.

MAMMALIAN FUNCTIONAL GENOMICS

A. Mills A. Bagchi W. Keyes
E. Garcia C. Papazoglu
X. Guo Y. Wu

The Mills laboratory designs and utilizes novel models for elucidating the genetic and molecular basis of human disease. Research areas include (1) the functional identification of novel tumor suppressor genes mapping to the short arm of human chromosome 1 and (2) investigation of the role of the p53-related protein p63 in development, cancer, and aging.

MOUSE MODELS OF HUMAN CANCER

A very exciting project in our lab has been the generatation of a series of mouse strains harboring chromosome 4 deletions in an attempt to functionally identify novel tumor suppressor(s) mapping to the corresponding region of the human genome---the short arm of chromosome 1. Briefly, chromosome engineering combines the power of gene targeting with Cre/*loxp* recombination and allows the generation of mouse strains that harbor precise deletions, which if they were to encompass a genomic interval to which a tumor suppressor maps, and a second hit were to inactivate the remaining functional copy of the suppressor, would provide a mouse model of hereditary cancer that could be used to design new anticancer therapies. The ultimate goal would be to identify the tumor suppressor within that region. A great advantage of chromosome engineering is that duplications corresponding to the deletion interval can also be generated, providing powerful genetic tools. By generating mouse models of hereditary human cancer, we hope to identify novel tumor suppressor genes.

FUNCTION OF THE *p53* HOMOLOG *p63*

p63 ***Deficiency Is Tumor Protective.*** The discovery that the *p53* tumor suppressor is a member of a multigene family that also includes *p63* and *p73* has brought the *p53* field into a new era. *p63* is a transcription factor structurally and functionally similar to *p53*; in contrast to *p53*, however, *p63* is rarely inactivated in human cancers. In fact, the *3q27* region to which *p63* maps is frequently amplified, or *p63* is overexpressed in the majority of epithelial tumors, suggesting that *p63* has oncogenic potential. Several years ago, we identified *p63* and generated several *p63*-deficient mouse models. What was clear from the phenotype was that despite the striking similarities between *p63* and *p53*, they perform very different functional roles in vivo: *p63* is essential for development of stratified epithelia, whereas *p53* is dispensable during embryogenesis but functions as a potent tumor suppressor in the adult. But does p63 also function as a tumor suppressor? We addressed this question by determining whether $p63^{+/-}$ mice were cancer-prone. In contrast to the high incidence of spontaneous tumors in $p53^{+/-}$ mice, $p63^{+/-}$ mice were not predisposed to cancer. In fact, *p63* heterozygosity decreased the high tumor incidence of $p53^{+/-}$ mice, indicating that haploid levels of *p63* may even be tumor protective. Loss of the wild-type *p63* allele did not occur and *p63* expression was maintained in the rare tumors that did develop in the $p63^{+/-}$ cohort. Given p63's essential role and robust expression in stratified epithelia, we examined whether chemical carcinogens applied directly to the skin might reveal a tumor predisposition that was not appreciated by assessing spontaneous tumorigenesis. However, $p63^{+/-}$ mice were not susceptible to chemically induced tumors. Indeed, $p63^{+/-}$ mice developed similar numbers of papillomas, the same percentage of these progressed to squamous cell carcinomas, and *p63* expression was maintained. This work reveals that *p63* does not perform a role equivalent to p53 in tumor suppression and that reduced p63 may provide a novel tumor suppressive mechanism, suggesting that modulation of p63-mediated pathways could offer an effective strategy for anticancer therapy.

p63 ***Links Cellular Senescence and Aging.*** During the course of the tumor study outlined above, we discovered that $p63^{+/-}$ mice had a significant reduction in life span and developed age-related pathology. To investigate the role of *p63* in the adult, we generated a conditional system in which *p63* could be disrupted in a tissue-specific and -inducible manner. This model circumvents the neonatal lethality imposed by *p63*

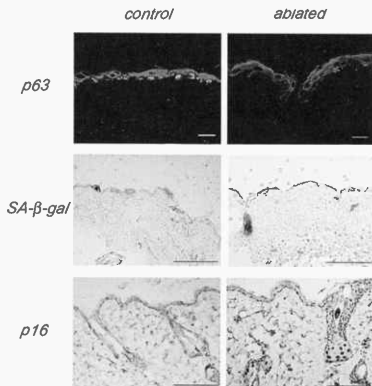

FIGURE 1 Induced loss of p63 in adult skin causes cellular senescence. Eight-month-old mice were treated with inducer to activate *Cre*. *Cre*-mediated recombination disrupts the *p63* locus and efficiently ablates p63 protein (*upper panels*) in *p63flox/flox/K5CrePR1* [*ablated; right*]), but not in control (*left*) skin. *p63* ablation induces markers of cellular senescence such as senescence-associated-β-galactosidase (SA-β-gal; *middle panels*) and p16 (*lower panels*). These results indicate that *p63* modulates the senescent process in vivo.

deficiency; phenotypically "normal" mice are generated, and Cre is activated specifically within stratified epithelia such as the skin by treatment with inducer at the desired time point. This system efficiently disrupts *p63* and ablates p63 protein in vivo—during embryogenesis, in the neonate, and in the adult. In addition, this model provides a relevant cell-based system to study p63 function: primary keratinocytes. To date, we have used this system to reveal an unanticipated link among p63, cellular senescence, and aging. p63 deficiency activates a program of cellular senescence and leads to accelerated aging. Both germline-induced and somatically induced p63 deficiency causes up-regulation of senescence markers SA-β-gal, promyelocytic leukemia (PML), and *p16* in the embryo. When *p63* is ablated in adult mice, the aging process is dramatically accelerated, and markers of cellular senescence are up-regulated (Fig. 1). Although p63 deficiency-induced cellular senescence in the germ line is *p53* independent, senescence is prevented in Cre-infected primary keratinocytes by short-hairpin RNA interference (shRNAi)-mediated knockdown of p53 or PML, but not by knockdown of p16. The finding that p63 deficiency activates cellular senescence suggests a mechanism for the low tumor incidence of $p63^{+/-}$ mice: cellular senescence effectively removes aberrantly proliferating cells from the proliferative pool, thus providing a tumor-suppressive mechanism. A further understanding of how *p63*-mediated pathways modulate the senescent process will impact our ability to design more effective anticancer regimens in the future.

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ANGIOGENESIS-MEDIATED TUMOR GROWTH AND METASTASIS

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B. Cong A. Mellick
D. Gao D. Nolan
A. Jadali R. Stephen

Neovascularization is a distinguishing hallmark of cancer malignancy. The induction of tumor vasculature, termed the "angiogenic switch," is critical for tumor progression and metastasis. Proangiogenic growth factors such as VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor), angiopoietins released by tumor cells promote activation, proliferation, and migration of endothelial cells (ECs) to tumor tissues, allowing for rapid formation of functional neovessels. Thus, targeting angiogenesis in tumors is a promising anticancer therapy. Indeed, the first antiangiogenic cancer drug—the anti-VEGF antibody Avastin—has provided an overall survival benefit in colorectal, breast, and lung cancer patients when combined with conventional chemotherapy. Despite promising success, cancer patients receiving a single class of angiogenesis inhibitors, even in combination with chemotherapy, eventually succumb to their disease. Several possible mechanisms exist that may explain the acquired resistance of antiangiogenic drugs. For instance, continuous angiogenic block is often followed by a regrowth phase. These regrowing tumors display a more invasive and malignant phenotype associated with reinduction of angiogenesis and the reestablishment of tumor vasculature. It has also been suggested that the recruitment of bone marrow (BM)-derived proangiogenic cells may also provide a potential mechanism of escape to some antiangiogenic strategies. Thus, targeting ECs or the principal angiogenic factor VEGF alone may not suffice to eradicate malignant tumors, and other components, including the endothelial progenitor cells (EPCs), may need to be targeted as well.

Solid tumors are composed of malignant cells and many other nonmalignant cell types. This produces a unique microenvironment that can modify the neoplastic properties of the tumor cells. The tumor microenvironment contains many resident cell types, such as adipocytes, fibroblasts, and migratory hematopoietic cells, most notably macrophages, neutrophils, and mast cells. Tumor-derived angiogenic factors also promote recruitment of BM-derived hematopoietic stem/progenitor cells, endothelial progenitors, and pericyte progenitors. All of these cells have pivotal roles in the progression of primary tumors, as well as metastatic dissemination. Although the perivascular contribution of BM-derived hematopoietic cells in neovasculature formation has received much attention, little is known about the nature and function of BM-derived EPCs.

The focus of research in our laboratory is to understand how tumors build blood vessels by de novo recruitment of BM-derived EPCs and how we can exploit these cells in delivering and evaluating antiangiogenic therapy. In last year's Annual Report, we described the establishment of a BM transplantation (BMT) model to determine how tumors direct the expansion, mobilization, and incorporation of EPCs into the functional tumor neovasculature. We showed that BM-derived ECs had incorporated into functional neovessels in the tumor. We also determined up-regulation of transcription factors Id1 in tumor-challenged BM precursors. Consequently, short hairpin RNA (shRNA)-mediated suppression of *Id* gene expression resulted in a significant delay in tumor growth as a consequence of vascular defects. We have now expanded upon the critical role of EPCs further by (1) defining their contribution to vessel formation at various stages of tumor growth, (2) understanding the molecular mechanisms that govern EPC mobilization, and (3) determining their broader role in neovascularization of tumors in the metastatic sites.

To Identify, Characterize, and Determine the Contribution of EPCs in Tumor Neoangiogenesis

D. Nolan, K. Bambino, B. Cong, A. Jadali [in collaboration with D. Scheinberg and R. Benezra, Memorial Sloan-Kettering Cancer Center, New York]

We have continued to exploit the BMT model further to determine the contribution of BM-derived EPCs in neovessel formation at various stages of tumor growth. Early tumors showed a marked recruitment of BM-

derived EPCs at the periphery of the advancing tumor margins. These cells represent EPCs as judged from their uniform cell surface expression of VE-cadherin, low CD31 (greater than tenfold), VEGFR2, and stem cell marker Prominin I. These cells were distinct from hematopoietic lineages as they did not express markers such as CD11b (monocytes, macrophages, granulocytes), CD45RB (monocytes, granulocytes, subsets of T and B cells), and CD41 (megakaryocytes). Analysis of later tumors showed a reduction in the number of EPCs at the tumor periphery and recruitment of $CD31^+$ mature blood vessels. These vessels had luminally incorporated BM-derived green fluorescent protein (GFP) mature ECs. We quantitated luminally incorporated BM-derived EC in tumor vessels. Approximately 17% of all vessels had incorporated BM-derived ECs, and these vessels markedly decreased with tumor growth. We also performed systemic perfusion with fluorescently labeled isolectin IB4, which specifically stains distinct oligosaccharides of the ECs in functional blood vessels. Flow cytometry analysis showed that approximately 8% of ECs in orthotopic melanomas and about 18% in LLCs were BM-derived ECs. Although the recruitment of BM-derived ECs was observed persistently in the tumor vasculature, the notion that EPCs are required for angiogenesis-mediated tumor growth needed formal substantiation. To determine this, we directly ablated EPCs with the VE-cadherin function-blocking antibody E4G10. Importantly, E4G10 recognizes the exposed monomeric epitope of VE-cadherin that has not yet engaged in *trans*-adhesion observed in mature ECs in vessels. Therefore, E4G10 does not target normal vasculature or tumor vasculature but recognizes the monomeric VE-cadherin present on EPCs. To increase the potency of E4G10 at low concentrations, we conjugated it to radioactive actinium-225 (^{225}Ac). Administration of ^{225}Ac-labeled E4G10 at days 3, 5, 7, and 10 significantly reduced the accumulated tumor burden per animal (by ~50%, compared to the administration of ^{225}Ac-labeled IgG isotype control [Fig. 1a]). The impaired tumor growth was associated with a marked reduction (>45%) in VE-cadherin$^+$ EPCs (Fig. 1b). This effect was specific because no

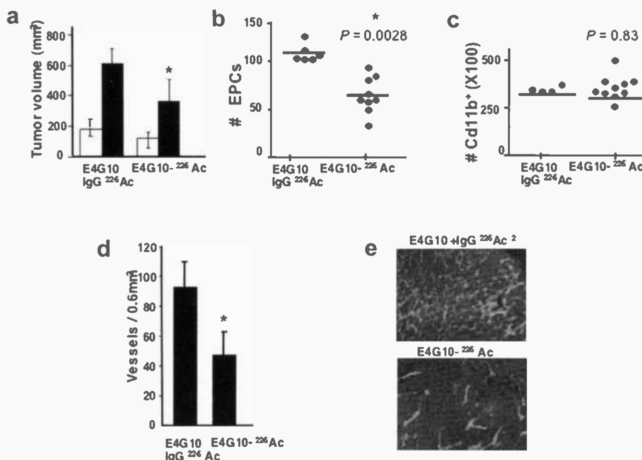

FIGURE 1 Ablation of endothelial progenitor cells impacts tumor growth in vivo. Representative sections are from 3 to 6 mice/group. Error bars represent standard deviations. Asterisks represent significant by *t*-test. (*a*) Lewis lung carcinoma tumor volume at day 6 (*white box*) and day 14 (*black box*) in animals administered with control ^{225}Act-labeled IgG or ^{225}Act-labeled E4G10 antibody (n = 8). (*b*) BM-derived GFP$^+$ VE-cadherin$^+$ CD31$^-$ CD11b$^-$ cells in early tumors in animals administered with control ^{225}Act-labeled IgG or ^{225}Act-labeled E4G10 antibody (n = 6–9). (c) CD11b$^+$ cells in animals treated as in *c* (n = 10 mice). (*d*) Vessel density in tumors in animals treated with control and test antibody (day 14). (e) CD31 immunostaining of tumor sections (day 14).

detectable change was observed in the CD11b$^+$ hematopoietic cells among ^{225}Act-labeled E4G10, ^{225}Act-labeled IgG, or untreated animals (Fig. 1c). Thus, ablation of VE-cadherin$^+$ EPCs resulted in marked delay in tumor growth as a consequence of vascular defects (Fig. 1d,e).

We are currently identifying pure EPC populations resident in total BM, dissecting the role of the *Id* pathway, and exploiting EPCs to serve as cellular vehicles for delivering effective therapeutics in the form of (1) DNA-encoded inhibitory shRNAs, targeting proangiogenic genes (e.g., components of the *Id* pathway) and (2) other therapeutic cargos (e.g., suicide genes and antiangiogenic gene products) directly and specifically to the sites of the tumor neovasculature to inhibit its growth and metastasis.

To Determine Transcriptional and Posttranscriptional Events Regulating Mobilization of EPCs

A. Mellick [in collaboration with E. Turner, University of California, San Diego]

The molecular pathways that determine EPC mobilization/differentiation are largely unknown. We have used DNA microarray technology to identify candidate genes and endogenous microRNAs (miRNA) that mediate mobilization of EPCs in response to tumor challenge.

Gene expression changes were identified in freshly isolated Lin$^-$ BM progenitors from tumor-challenged animals at days 0, 4, 6, and 9 using DNA microarrays. Of the 14,000 genes examined, on average, 150 of the transcripts were found to be differentially regulated by at least 1.5-fold. For validation by real-time quantitative polymerase chain reaction (Q-PCR), we selected candidates whose expression profiles followed the clearance (days 4–6) profile of VE-cadherin$^+$ cells from the BM, as these might be particularly indicative of early EPC-specific changes (occurring in the BM) in response to tumor challenge. In this way, certain candidate genes, such as helix-loop-helix transcription factor *Id1*, chemokine receptor CXCR4, stroma-derived factor SDF1, VE-cadherin, flk1/VEGFR2, GATA2, and Brn3a, were identified. The expression of some of these candidate genes was localized to the Lin$^-$ VE-cadherin$^+$ EPCs.

To determine posttranscriptional events that regulate EPC mobilization, we have developed a system for identifying the differential expression of all known mouse, human, and rat miRNA. To determine miRNA changes that may regulate EPC mobilization, we isolated miRNAs from freshly harvested BM Lin$^-$ progenitors from tumor-challenged animals at days 0, 4, 6, and 9. Each miRNA sample was labeled with Cy5 or Cy3 and hybridized to the array. Quantitative changes in miRNA expression showed that 53 miRNAs were differentially regulated (at least twofold) in at least one time point in the Lin$^-$ BM, following tumor challenge. All regulated miRNAs with unknown mRNA targets were analyzed by computer-based target prediction algorithms. We selected miRNAs whose expression profiles followed the clearance (day 6) profile of VE-cadherin$^+$ cells from the BM, as these might be particularly indicative of early EPC-specific changes in response to tumor challenge. We are currently investigating the role of these candidates in EPC mobilization.

To Determine the Role of EPCs in the Progression of Avascular Micrometastases to Vascularized Macrometastases

D. Gao

The progression of micrometastases into vascularized macrometastases is the leading cause of mortality in cancer patients. However, not much is known about the regulation of the antigenic switch or the dynamics of vessel assembly during this progression. To this aim, we will investigate the role of EPCs in neovascularization of metastatic lesions. It is conceivable that targeting EPCs may be a more clinically appropriate therapeutic approach in blocking growth of metastatic lesions as compared to primary tumors. This is because in many cases, by the time a primary tumor is detected, EPC-mediated neovascularization has already occurred.

We have developed a system for tracking both BM-derived cells and tumor cells in vivo, so that these cells can be localized in the metastases in secondary organs. Tumor cell lines, including LLCs and an isogenic pair of metastatic (B16F10) and nonmetastatic (B16F0) melanomas, stably expressing monomeric red fluorescent protein (RFP, cherry version), were generated. Clonal tumor cell lines were derived, propagated, and tested for their ability to form metastases. Colonies that expressed high levels of RFP and successfully formed metastases were used in subsequent

experiments. To determine the contribution of BM-derived EPCs to the formation of neovasculature in the metastatic colonies, we challenged GFP^+ BM reconstituted mice intradermally with either RFP-expressing LLCs, which metastasize to the lungs and occasionally the liver, or B16 melanoma cells, which possess a more widely disseminated metastatic potential. The primary tumors were allowed to grow for 2 weeks (average size 300 mm^3) and were then resected to control morbidity associated with excess primary tumor burden, which would otherwise have occurred at later time points. We observed the appearance of micrometastases (average 2–5 per animal, ~0.5 mm in diameter) at the time of primary tumor resection (day 14). Macrometastases were visible after 1 week. Notably, in most metastases, GFP^+ BM-derived cells colocalized with the tumor cells, indicating the importance of these BM-derived cells in the growth of these metastatic lesions. Metastasis was also successfully tracked in melanoma cells implanted orthotopically in the skin. Histological analysis on these tumors to determine the status of their vasculature showed that the early tumors were avascular as determined by the absence of $CD31^+$ vessels in the tumor bed. Analysis of macrometastases (days 21–28) showed the recruitment of $CD31^+$ mature vessels, indicating that these colonies had undergone an angiogenic switch. Notably, some of the early-stage micrometastases showed recruitment of BM-derived EPCs. Analysis of tumor vasculature showed incorporation of BM-derived GFP^+ $CD31^+$ mature ECs in macrometastases. We are expanding upon these observations further, by quantifying EPC contribution, and determining their functional role in angiogenesis-mediated tumor growth. We will next determine the consequences of blocking EPC function on the progression of these metastatic lesions. As a first line approach, we will use the VE-cadherin-blocking antibody E4G10, which specifically targets the monomeric VE-cadherin epitope. In parallel, EPC function will be blocked by shRNAs-mediated conditional suppression of *Id1* and VE-cadherin.

Kathryn Bambino

CANCER GENES

S. Powers A. Bakleh D. Mu
R. Kohnz K. Nguyen
C. Kuscu

This year marks the first full year for our laboratory, which we set up in October of 2004. Our broad interest is to identify, validate, and acquire new biological or clinical knowledge about human cancer genes. We are committed to perform studies that can benefit cancer treatment in the near-term, either by validating a new cancer therapeutic target or by developing biomarkers than can be used to guide treatment. Along these lines, this year, we engaged in a productive collaboration with Scott Lowe's lab that led to the development of a new and powerful integrative approach to cancer gene discovery and validation. This approach uses comparative genomic analysis of human and mouse model cancers. Key to this project was the implementation of the highly innovative representational oligonucleotide microarray analysis (ROMA) technology, and its associated informatics tools, developed over the past years at CSHL by Mike Wigler, Rob Lucito, and key people in Wigler's informatics group. Using this approach, we discovered and validated this year a new human oncogene operative in hepatocellular carcinoma, a highly lethal cancer that is neglected by the pharmaceutical industry due to its Third World status. Rebecca Kohnz, a Stony Brook graduate student, started this year and is working jointly on this project with Lars Zender, who developed the hepatocellular carcinoma (HCC) mouse model in Scott Lowe's lab, along with key collaborators Mona Spector, Wen Xue, and David Mu. We are excited about a recently uncovered additional oncogene amplified in both human and mouse HCC that we hope will lead directly to the development of a new monoclonal antibody treatment.

Another collaborative project, headed by Greg Hannon's group, led to the definitive functional validation of a human oncogene encoded by a microRNA, a new class of genetic regulators. This was the first strong indication that microRNAs can have a substantial functional role in cancer, and it was published alongside a surprising report that microRNA expression profiling is a more powerful discriminator of tumor types than mRNA profiling.

Following up on last year's ROMA-based discovery that colorectal cancers have frequent focal deletions, this year, we discovered that the most common deletion in human colon tumors, present in approximately 20% of tumors, affects a specific splicing factor gene. Of the remaining 80% of colon tumors, one half of them have an alternative mechanism for suppressing expression of this gene. Thus, dysregulation of this gene affects the majority of colorectal cancers. Cem Kuscu, a graduate student from Stony Brook, recently obtained preliminary functional data which indicate that this gene is a bona fide tumor suppressor. It is possible that alternatively spliced proteins caused by this deletion will provide selective targets for cancer drug development, and we are collaborating with Adrian Krainer's laboratory to identify these targets. This work dovetails with the prior collaboration David Mu had with the Krainer lab concerning the largely understudied role of dysregulated alternative splicing factors in cancer progression.

This year, we began a project in colon cancer that runs parallel to breast cancer efforts in Mike Wigler's lab to utilize genomic profiles of human tumors to predict future clinical outcome and response to different treatments. Here, we aim to meet the challenge of developing DNA-based diagnostic tests to guide treatment choices for individual cancer patients. Although it is clear from our results that recurrent, metastatic colon cancers on average have more DNA-copy-number alterations than nonrecurrent curable colon cancer, we have yet to determine whether or not there are specific loci associated with metastasis.

Finally, David Mu has completed a ROMA project where he has analyzed approximately 250 human lung cancer samples. He found that the number of distinct focal loci (<5 Mb) affected by either high-level amplification or deletion is more than 500, well over the current estimates of 300 human cancer genes. Remarkably, more than 300 of these loci occur only once, and only a handful of amplicons and deletions have frequencies above 5%. Although it is easy to dismiss these rare amplicons or deletions as background noise generated by the genomic instability of tumors, there are several validated human cancer genes within this group. David is currently using short hairpin RNA (shRNA) constructs from the CSHL RNAi Codex library to address the functional significance of rare

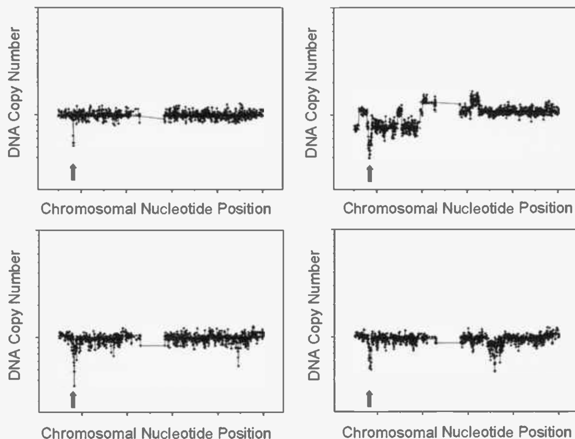

FIGURE 1 Frequent deletion of the alternative splicing factor gene in colorectal tumors as detected by ROMA analysis.

amplicons. It could be that a much larger degree of diversity exists in the genomic alterations that can contribute to cancer than previously realized.

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GENE COPY-NUMBER VARIATION AND ITS ROLE IN HUMAN DISEASE

J. Sebat J. Lloyd
D. Malhotra
S. Yoon

Large-scale differences in gene copy number called copy-number polymorphisms (CNPs) are a significant source of human genetic variation. In contrast to DNA sequence variants such as single nucleotide polymorphisms (SNPs) and microsatellite repeats, CNPs have not been well-characterized. We seek to understand more about the genomic locations, frequency, and stability of these structural variants and their role in human disease.

Determining the Genetic Basis of Autism

J. Sebat, S. Yoon, D. Malhotra [in collaboration with M. Wigler, K. Ye, B. Lakshmi, A. Krasnitz, and B. Yamrom, Cold Spring Harbor Laboratory]

Autism is a neuropsychiatric disorder characterized by deficits in social interaction and communication and unusual and repetitive behavior. Autism affects an estimated 1 in 250 births, and its prevalence is increasing. The causes of autism are poorly understood. On the basis of the results of twin studies, it has been clearly determined that genes have a major role in the etiology of the disease, but genetic studies have succeeded in identifying very few good gene candidates. To make further progress in understanding the genetic basis of autism, it is critical to use novel methods that are complementary to traditional genetic approaches. We have developed a novel approach for directly identifying the underlying mutations that cause autism: high-resolution analysis of gene copy number. Our approach uses a method developed by Rob Lucito and Mike Wigler called representational oligonucleotide microarray analysis (ROMA). We are using ROMA to test the hypothesis that alterations in gene dosage are a basis for heritable and sporadic neuropsychiatric disorders.

Our data provided compelling evidence that submicroscopic alterations in gene copy number are important in autism. ROMA analysis of 110 sporadic cases of autism (and parental DNAs from a subset of families) has identified six mutations that occurred spontaneously in the child (see the example in Fig. 1). Five of these variants have been previously reported in association with autism. We identified one novel variant that consists of a deletion of chromosome 20p13. The deleted region contains multiple genes potentially involved in development, some of which are predominantly expressed in the central nervous system. In an attempt to identify inherited copy-number variants that cause autism, we screened a second sample of approximately 150 patients from families with multiple affected siblings. No spontaneous mutations were identified in these patients, which is consistent with the notion that the genetic factors here are more likely to be inherited. Inherited variants were identified that are more frequent in autism than in controls. We have typed several of these inherited variants in affected and unaffected family members and determined a subset that segregate with autism in small families.

We have succeeded in identifying novel candidate genes by detecting spontaneous mutations in patients with sporadic autism. Further characterization of these novel autism candidate genes may yield insight into the processes of brain development that are impaired in autism. By expanding our analysis of sporadic autism, we hope to discover additional causative mutations. Second, to more thoroughly evaluate the association of inherited copy-number variants with autism, we will screen a larger sample of families with multiple affects.

Analysis of Large-scale Copy-number Variation in the HapMap

J. Sebat, S. Yoon, J. Lloyd, D. Malhotra [in collaboration with K. Ye and B. Lakshmi, Cold Spring Harbor Laboratory]

Much remains to be learned about the extent of "normal" structural variation in the human genome and its implications for genetic studies of populations. We have initiated a CNP discovery effort that will focus on DNA samples from the International HapMap Project. The key advantage of using the HapMap samples for

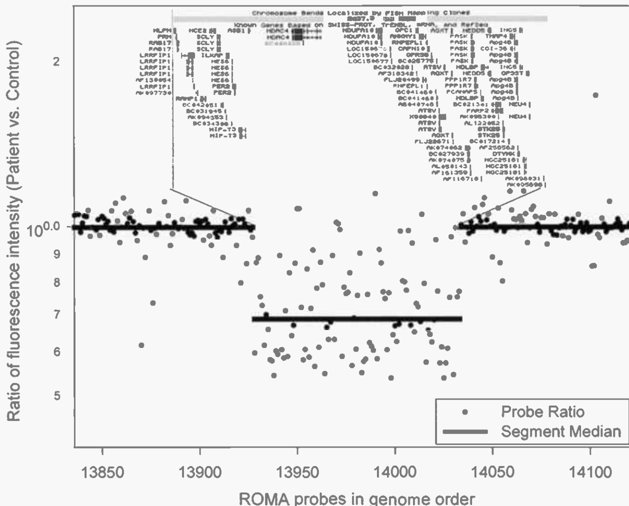

FIGURE 1 A submicroscopic deletion of chromosome 2q37 was detected in a patient with autism. ROMA analysis of DNA from both parents revealed that this deletion occurred spontaneously in the child. Data points represent the fluorescence ratio measured by individual ROMA probes. Known genes present within the deleted region are shown.

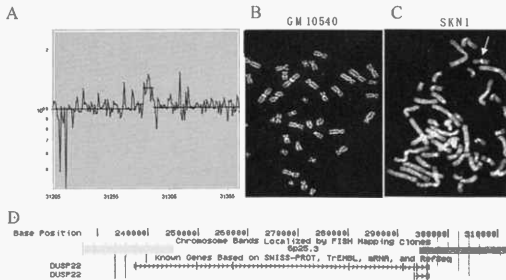

FIGURE 2 *DUSP22* locus selected for analysis of SNPs in linkage disequilibrium with CNPs. (*A*) ROMA data showing a 4:3 difference in copy number between two individuals. (*B*,C) FISH (fluorescence in situ hybridization) results on the same two individuals confirm the presence of four and three copies, respectively, the polymorphic allele occuring at 16q11. (*D*) Display of the amplified genomic region.

this study is the availability of high-density SNP genotypes on the same individuals. CNP data can be combined with SNP data to determine, for example, whether a polymorphic duplication or deletion is associated with a unique SNP haplotype (indicating that the variant results from an ancestral event that was inherited by descent) or with many different haplotypes (suggesting recurrent structural mutation at that site). In addition, direct analysis of copy-number variation is helpful for resolving aberrant patterns in the HapMap data that result from CNPs, such as apparent non-Mendelian patterns of inheritance. Here, we analyzed copy-number variation in 30 CEPH trios using ROMA.

In an initial attempt to integrate a structural variant into the HapMap, we analyzed linkage disequilibrium between SNPs and a single CNP identified by ROMA: duplication of the dual specificity phosphatase 22 gene, DUSP22 (Fig. 2). We genotyped each individual as having two, three, or four copies of the gene, corresponding to genotypes of –/–, +/–, and +/+ for the duplicated allele. We then tested association with all HapMap markers across the genome and identified four markers showing a very strong association with this CNP (LOD = 7.4–9.7). These markers were mapped to within the DUSP22 gene itself, thus validating our computational approach to defining the SNP haplotype on which a given CNP resides. In addition, we used publicly available mRNA expression data to examine the expression of DUSP22 in 37 HapMap individuals to determine the effect of copy number on DUSP22 expression in lymphoblasts. There was a positive correlation between copy number and expression that is statistically significant ($p = 0.03$). These data illustrate how our results may help create new opportunities for researchers to apply computational approaches to existing data to understand biological significance of this newly discovered type of human variation.

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Jobanputra V., Sebat J., Troge J., Chung W., Anyane-Yeboa K., Wigler M., and Warburton D. 2005. Application of ROMA (representational oligonucleotide microarray analysis) to patients with cytogenetic rearrangements. *Genet. Med.* **2:** 111–118.

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COPY-NUMBER ANALYSIS AND HUMAN DISEASE

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Our studies of cancers and leukemia center around a technology developed in this lab called representational oligonucleotide microarray analysis (ROMA). ROMA is a high-resolution, high-throughput method for detecting copy-number changes in the genome, such as amplifications and deletions characteristic of the mutations that drive malignancies. These changes are manifest as alterations in the genome "profile" of a cancer or leukemic cell.

GENOMIC ANALYSIS OF CANCER AND LEUKEMIA

Breast Cancer. In a long-standing collaboration with Anders Zetterberg at the Karolinska Institute in Stockholm, Sweden and Anne-Lise Børresen-Dale at the Norwegian Radium Hospital, University of Oslo, Norway, we have profiled more than 250 breast cancer tumors from separate collections of Norwegian and Swedish populations. We have reached several important conclusions. First, we observed a type of genomic rearrangement that we call "firestorms"—regions of chromosomal instability localized within chromosome arms—and we have determined which chromosomal regions are prone to this destabilization. Even a single firestorm is an especially significant prognostic marker of poor outcome. Second, the number and spacing of genomic lesions correlate very strongly with survival. We derived a mathematical measure that captures the essential geometric features of the profiles that correlate with survival in the population of pseudodiploid cancers ($p = 10^{-7}$). This is a far stronger correlation than is obtained from knowledge of any particular locus and may subsume any locus-based predictive determination. The information in this measure is independent of the location of amplifications and deletions, and of classical clinical parameters such as node status, stage, and expression of hormone receptors. Our measure will thus have clinical utility in patient evaluation.

Our data from the above studies also help to delimit the location of loci of oncogenes and tumor suppressor genes. We have worked out a variety of probabilistic and heuristic methods that sum over the data set that mark what we believe to be the most likely locations for cancer genes. We observe a similar set of loci whether examining pseudodiploid or aneuploid cancer subpopulations, even though the latter have many more lesions, suggesting that these loci confer advantage to the tumor, regardless of the degree and type of genomic instability.

The overall similarity in the location of regions of genome instability in breast cancers in Swedish and Norwegian populations is striking. These data provide a baseline from which we can seek differences in individuals of other ethnic backgrounds and geographic locations. Such comparative studies might reveal the extent of genetic and environmental contributions to the initiation and development of breast cancer or, for that matter, any cancer.

Mouse Models of Human Malignancy. In a collaboration with Robert Lucito here at CSHL, we have used our experience in designing a human ROMA copy-number array to design and test a mouse ROMA chip. This array can be used to study the amplifications and deletions that occur in mouse tumors, and that data can be compared to profiles of similar tumor types in humans. In another collaboration with Scott Powers and Scott Lowe's labs here at CSHL, this approach has been used successfully to define a common gene amplified in mouse and human liver cancers, which encodes an inhibitor of apoptosis. The mouse-human synteny relationships are used to define the common genetic elements in amplified or deleted loci. As a general approach, the profiling of human cancer genomes and the analysis of mouse models of human cancer is a powerful combination for defining the causes of this disease.

In a collaboration with David Botstein and Robert Pelham of Princeton University, New Jersey, we have used mouse ROMA to examine the "normal" stroma that grow in response to implantation of a human cancer cell line in mice. We detect clear evidence that stroma have clonal subpopulations that carry small deletions and duplications. Although preliminary, the

implications could be profound: If tumors cultivate or recruit mutant stroma, a vulnerability in the cancer-host communications could be potentially disrupted, leading to novel therapeutic approaches.

Leukemia. In a collaboration with Nick Chiorazzi at North Shore University Hospital in Manhasset, New York, we have initiated a study of chronic lymphocytic leukemia (CLL), the major form of lymphoma/leukemia in adults. We use ROMA to examine copy-number changes in leukemic cells, using normal blood neutrophils from the same patient as a control. We have completed a series of 21 patient samples. In summary, 10/21 samples have a 13q deletion, and five of these deletions are homozygous. The epicenter spans DLEU7 but not mir-15a or mir-16-1, the micro RNAs (miRNAs) that others have proposed to be in the epicenter, throwing into question the validity of the hypothesis that expression of these miRNAs are important in this form of leukemia. In addition, 3/21 samples show multiple narrow deletion events and define a new class of chromosomal instability in CLL, which is not, to our knowledge, previously reported. Two new loci are the recurrent target of small-sized events, one at a gene proposed to be involved in membrane trafficking and one at a locus containing no known genes.

ANALYSIS OF HUMAN GENETIC DISEASE

Our studies of human genetic disease likewise center around ROMA. In general, with ROMA we can observe two types of phenomena: (1) spontaneous mutation that results in a copy-number change in an afflicted human and (2) an inherited polymorphism of copy number that affects the likelihood or severity of a disease. Neither of these genetic events were readily detectable by prior methodology, so ROMA, or an equivalent copy-number measurement technique, opens new possibilities for understanding forms of human disease with strong genetic components. Our particular interests lie in autism and other neuropsychiatric disorders of early onset, in a collaboration with Jonathan Sebat of CSHL; congenital heart disease with Dorothy Warburton and Wendy Chung of the Columbia UniversityCollege of Physicians and Surgeons, New York; and familial prostate cancer, in a collaboration with Bill Isaacs of Johns Hopkins University School of Medicine, Baltimore, Maryland.

As a baseline for all of these studies, we must analyze the "normal" variation of the human genome. This baseline catalog of copy-number polymorphisms (CNPs) is necessary to distinguish novel events that might be associated with disease from preexisting events that are part of normal human copy-number variations. Currently, we have identified approximately 510 unique CNPs from 500 individuals.

Distinction between Sporadic and Inherited Autism. We have established a distinction between sporadic and familial autism, in particular, we established the role of de novo mutation as a cause of autism in the former, as we originally postulated. In 90 samples from families with one autistic child but with no other history of the disease, we have observed six confirmed examples of spontaneous deletion or duplications. There may be additional examples of these, much smaller in size, that await confirmation by higher-resolution ROMA. In contrast, in the AGRE (Autism Genetic Research Exchange) set of 170 patients who derive from families with two or more affected children, we see only one possible example of a spontaneous mutation. This is on the X chromosome and is found in both affected children, but not in the parents.

We have limited data on the rate of spontaneous amplifications/deletions in normal family trios, but in 30 family trios we have found no clear examples of new mutations. We need to solidify these observations with more rigorous standards and more data, but our findings point to the conclusion that sporadic autism can result from spontaneous mutation.

This observation has an important implication for how the community of genetic researchers proceed in the discovery of mutations that cause autism. Trios of mother, father, and child, where there is no other affected child or history of autism in the family, may be the preferred population in which to search for spontaneous mutations. Unfortunately, most of the community's effort has been directed to the collection of families with more than one affected child, where transmission genetics has a greater role.

Protective Variation in Autism. In familial autism, we have preliminary evidence for the possible involvement of relatively common alleles in the penetrance of the disorder. In particular, our data suggest the involvement of the *CHRNA7* locus encoding the nicotinic acetylcholine receptor α7. This locus is of interest because of its suspected involvement in schizophrenia and attention deficit disorders. Our data indicate that this region is hypervariable in the human population and may thus be under strong selective pressure. Duplication at this locus is observed in individuals from all populations, including sporadic cases of autism, at rates about 15%, but in the AGRE set, the

frequency of duplication is much lower, around 5%. These results suggest that the presence of duplication at this locus might protect against autism of the inherited variety.

This hypothesis is important in several respects. If correct, it suggests that in some cases, pharmacological approaches might alleviate development of the disorder. Second, it may provide mechanistic insight into the nature of the disease. Third, the hypothesis raises hope that other genetically linked disorders could yield to similar approaches. Important hypotheses require extremely careful analysis, and far more work than what we have completed thus far is needed to confirm this observation.

ADVANCES IN ROMA TECHNOLOGY

ROMA offers a fresh perspective on human disease, but its potential is still largely untapped. Improvements to the methodology in terms of resolution, reliability, data access, and cost are a continuing and challenging activity within our group.

New Designs and Applications. We have successfully developed and tested a high-resolution 390,000 probe ROMA chip, with more than four times the probe density of our previous 85,000 probe chip. With this microarray, we can see cancer lesions with far greater clarity than before, which should aid greatly in determining the location of genes causing cancers and leukemias. Work remains to modify the processing software that we have developed for the 85,000 array, including normalization and segmentation protocols. (Normalization is a method for standardizing interpretation of raw hybridization signals, and segmentation is a method for interpreting the hybridization data as alterations of chromosomal loci.) We have also developed methods that should enable the calibration of each probe on the array, which we predict will dramatically improve our segmentation methods and also help in selecting probes as we design new arrays in the future.

Additional statistical methods now allow us to perform hybridizations in single color, minus a reference sample. The ability to perform hybridization in one color, the omission of a reference sample, and the ability to reuse arrays after high-stringency washing reduces the consumption of arrays and other reagents and reduces costs almost twofold. Finally, we have established that we can obtain reliable data from tissue freshly fixed in formalin, which is the way in which breast and most other cancer biopsies are routinely prepared for clinical-pathological appraisal. All of these developments should facilitate the adoption of our methods in a clinical setting.

Data Access. ROMA generates raw data files on the order of megabytes per sample, and each sample is associated with critical biological information. The "accessibility" of our data has been a nagging problem. We have assays on over 2000 samples in our collective group (cancer and genetics), and each sample may have several dozen important associated clinical facts. Each assay may yield a table $40 \times (85 \times 10^3)$, and four times that for the high-resolution arrays. The raw data undergo a variety of processing steps and are stored in a variety of workspaces and formats distributed throughout the dispersed computers in four different geographic centers and two continents. Accessing all of this information in a seamless manner has been a trying experience. Our first implementation as a large centralized relational database of raw data was only a partial success, because it did not reference properly either sample information or the processed data files.

We have therefore taken two steps. The first is to implement an "Ark." The Ark contains all of the processed data files in a logical folder architecture on a central server as ASCII text, with specially designed "headers" in each file that identify and describe the internal structure of each file. Each folder contains an index to its contents in a standardized "language." We have developed tools for the rapid integration of this data back into the working directories of our numerical processing environment for comparative analysis, for graphical display, or as a jumping-off point to the Internet.

The second step has been to develop a customizable database language. It is a hybrid between object-oriented and relational database languages. Called "Pentuple," it uses something akin to natural language—a flexible linguistic structure with hierarchical permissions to alter that structure, allowing a user group to enter, organize, integrate, and search very large amounts of data. We hope that Pentuple will lead to a general purpose multiuser archival language system.

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Jobanputra V., Sebat J., Troge J., Chung W., Anyane-Yeboa K., Wigler M., and Warburton D. 2005. Application of ROMA (representational oligonucleotide microarray analysis) to patients with known cytogenetic rearrangements. *Genet. Med.* **7:** 111–118.

CANCER: CELL BIOLOGY

For a cell to multiply, it must first make an exact copy of its DNA to pass along to the two new cells. Bruce Stillman studies the molecular machinery inside the cell that coordinates and directs the process of DNA replication. First, the cell must "know" where to begin copying. DNA contains start sites called origins that direct the initiation of DNA replication. Binding to these origins are large complexes of proteins that have a role in the process. Recent work in the Stillman lab has revealed that two such proteins—Orc2 and Orc3—bind to centromeres or the "handles" on DNA through which chromosomes are pulled to the opposite ends of a cell before the cell divides. Stillman's group discovered that in the absence of Orc2 or Orc3, chromosomes either cannot duplicate or the duplicated chromosomes are improperly segregated, resulting in a loss or a gain of chromosomes as is often seen in cancer cells.

Arne Stenlund is using the papillomavirus replication system to gain a better understanding of the crucial first steps of how DNA is copied in human cells. He has managed to recapitulate the assembly of the viral "helicase," providing the first description of how such a protein-DNA complex is formed. The helicase is the enzyme that unwinds DNA in preparation for DNA replication. Until now, little has been known about precisely how this complex forms in human cells. Stenlund's efforts to dissect the helicase assembly process provides an opportunity to achieve a detailed understanding of the initiation of DNA replication in mammalian systems.

The body uses several families of proteins as biological "on/off" switches to control many processes. One such biological switch is the family of proteins that includes Rac and Rho. These proteins control a variety of important cellular activities including cell proliferation, cell-cell interactions, and cell signaling. In part of her work, Linda Van Aelst studies the role of Rac and Rho in the hippocampus, a part of the brain involved in the formation of new memories and the retrieval of older memories. Recently, she and her colleagues have identified a series of proteins that regulate Rac and Rho signaling and have shown that these proteins are required for proper nerve cell development and function in the hippocampus.

Nicholas Tonks is evaluating the links between a family of proteins called protein tyrosine phosphatases (PTPs) and human disease. Using information available from the human genome sequence, his group is studying the PTP "superfamily" of some 108 genes. Tonks and his colleagues are using RNA interference to uncover the function of each member of the PTP family. Initial studies include a collaboration with Senthil Muthuswamy to explore the function of the PTP superfamily in development of breast cancer.

Several investigators are beginning to find that cancer may arise in part from disruptions in tissue organization or the interactions among varying cell types within an organ. Muthuswamy studies the interaction between cancer cells and the surrounding tissue and structural proteins that organize breast tissue. He has developed a three-dimensional tissue culture system that mimics the basic glandular structure of the breast. Using this system, his group has identified a direct relationship between *ErbB2*, a gene known to be misregulated in nearly half of all breast cancers, and molecules that regulate cell architecture. *ErbB2* appears to inactivate proteins that help establish normal breast tissue structure. This ability of *ErbB2* to disrupt breast tissue organization may contribute to the observed disruption of normal tissue organization in breast cancer.

Tatsuya Hirano studies how cells organize and manage chromosomes during preparation for cell division. His current studies are focused on two protein complexes, called cohesin and condensin, that have key roles in compacting DNA and ensuring each daughter cell receives only one copy of each chromosome. The proteins belong to the SMC (structural maintenance of chromosomes) family, members of which are found in a wide variety of organisms from bacteria to humans. During the past year, Hirano studied SMC proteins of the bacterial species *Bacillus subtilis* as a model for more complex mammalian SMC proteins. His group showed that when the SMC protein first binds to DNA, the binding triggers a hinged section of the protein to move two swinging arms into place. These results help explain how SMC proteins control chromosome behavior.

David Spector and his colleagues have identified a messenger RNA that lingers uncharacteristically in the cell nucleus but can be rapidly released into the cytoplasm in response to cellular stress (or other signals) such as viral infection. The "cut-and-run" mechanism revealed by this study is a new paradigm of gene regulation that is likely to be broadly relevant to biology and biomedical research. It establishes a role for the cell nucleus in harboring mRNA molecules that are not immediately needed to produce proteins, but which are ready at a moment's notice to produce protein in response to stress or other cellular signals.

HIGHER-ORDER CHROMOSOME DYNAMICS

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R. Gandhi I. Onn
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The long-term goal of our research program is to understand the molecular basis of higher-order chromosome architecture and dynamics. Studies during the past decade have established that structural maintenance of chromosome (SMC) proteins are among the most fundamental regulators of chromosome dynamics conserved from bacteria to humans. In eukaryotic cells, SMC proteins constitute the core of two multiprotein complexes, condensin and cohesin, that have central roles in chromosome condensation and cohesion, respectively. In bacterial cells, SMC proteins function as part of a complex that also has a crucial role in chromosome segregation. Mutations in these SMC protein complexes lead to genome instability in many organisms. Moreover, emerging studies provide evidence that misregulation of condensin and cohesin might be linked to developmental diseases in humans. In our laboratory, we take multidisciplinary approaches to understand how condensin, cohesin, and SMC proteins might work at a mechanistic level both in vivo and in vitro.

Structural and Functional Dissection of Condensin I and Condensin II In Vitro

I. Onn, M. Hirano, T. Hirano

Previous studies from our laboratory have shown that vertebrate cells have two different condensin complexes, referred to as condensin I and condensin II. The two complexes share the same pair of SMC ATPase subunits (SMC2 and SMC4), but they contain different sets of non-SMC regulatory subunits. Despite clear demonstration that the two condensin complexes have critical roles in mitotic chromosome assembly and segregation in vivo, very little is known about how they might work at a mechanistic level in vitro. To address this fundamental question, we have used the baculovirus system to express recombinant subunits of condensin I and condensin II. Pairwise coexpression of individual subunits, followed by immunoprecipitation, allows us to construct a subunit interaction map for each complex. To refine specific interacting domains in each subunit, a panel of truncated proteins is constructed. We also reconstitute holo- and subcomplexes of condensins and assess the functional contribution of individual subunits to their DNA-binding, ATPase, and supercoiling activities. Furthermore, we prepare a panel of mutant complexes deficient in different stages of the SMC ATPase cycle and attempt to gain insights into the mechanochemical cycle of condensin I and condensin II. These efforts will undoubtedly enrich our mechanistic understanding of this amazing class of chromosome condensation machinery.

Dissection of Condensin Regulation in *Xenopus* Egg Extracts

N. Aono, I. Onn, T. Hirano

In *Xenopus* extracts, condensin I is loaded onto chromosomes in a mitosis-specific manner. To understand how this tight regulation of condensin loading is achieved, we currently focus on two specific questions: (1) What is the role of the ATPase cycle of SMC subunits in condensin loading? and (2) what is the contribution of individual non-SMC subunits and their phosphorylation in this process? To address the first question, we add back recombinant human condensin complexes containing either wild-type or mutant forms of SMC subunits into *Xenopus* egg extracts and test their ability to associate with chromosomes. We also test whether the added back fractions may functionally complement extracts depleted of endogenous *Xenopus* condensins. To address the second question, we add back individual recombinant subunits or subcomplexes and test their association with chromatin. Once basic information is obtained, the effect of mutations in potential phosphorylation sites will be tested.

A genetic study in fission yeast suggests that condensin I (the sole condensin complex in this organism)

may have a role in DNA repair and checkpoint responses. In vertebrates, condensin I is sequestered in the cytoplasm, whereas condensin II is located within the nucleus during interphase. We therefore hypothesize that condensin II, but not condensin I, may have a specialized role in DNA repair functions. This hypothesis is now being tested by asking how the two complexes may respond to damaged or undamaged chromatin templates in *Xenopus* egg extracts. Information derived from this line of experiments will also help us establish a comprehensive molecular picture of the loading and unloading mechanisms of condensins.

A Potential Link between Condensin Regulation and Microcephaly

T. Hirano [in collaboration with M. Trimborn, D. Schindler, and H. Neitzel, Institute for Human Genetics, Berlin]

Primary autosomal recessive microcephaly is a neurodevelopmental disorder characterized by marked reduction in brain size and mental retardation. Mutations in the *MCPH1* gene, encoding microcephalin, cause a unique cellular phenotype with premature chromosome condensation in early G_2 phase and delayed decondensation postmitosis. In collaboration with H. Neitzel's group in Berlin, we show that in *MCPH1* patient cells, small interfering RNA (siRNA)-mediated depletions of condensin II subunits lead to a pronounced reduction of cells with the condensation defects in both G_1 and G_2 phases of the cell cycle. Similar results are obtained when microcephalin and condensin II are simultaneously depleted in HeLa cells. In contrast, depletions of condensin I subunits do not reverse the cellular phenotype. Consistently, condensin I stays in the cytoplasm in the prophase-like cells of *MCPH1* patients. Our results offer a molecular explanation for the aberrant chromosome condensation in *MCPH1* deficiency and provide additional evidence that condensin I and II are regulated by distinct pathways.

Molecular Basis of Sister Chromatid Cohesion

K. Shintomi, P. Gillespie, T. Hirano

Although previous studies in many organisms have demonstrated that the cohesin complex is the key player in sister chromatid cohesion during mitosis and meiosis, very little is known about how it might work at a mechanistic level. To fully understand the molecular basis of sister chromatid cohesion, we have reconstituted holo- and subcomplexes of cohesin from its recombinant subunits by using the baculovirus expression system. We are now testing their behaviors and functions in *Xenopus* egg extracts. Mutant forms of cohesin will be included in this series of experiments to understand the role of the SMC ATPase cycle in cohesin functions. Another yet related aim of this project is to understand how establishment of cohesion might be coupled with DNA replication. To this end, we have devised a protocol for isolating the chromatin-bound fraction of cohesin from nuclei assembled in the extracts. By using various biochemical and immunological techniques, we plan to test whether cohesin might undergo conformational changes in a DNA replication-dependent manner or might recruit additional proteins during such a process.

Interplay of Centromeric and Arm Cohesion during Mitosis

R. Gandhi, T. Hirano

In vertebrate cells, cohesin is released from chromosomes by a two-step mechanism during mitosis. In prophase, most of the cohesin is released from chromosome arms, at least in part, by the action of two mitotic kinases, polo-like kinase (Plk1) and aurora B. A residual level of cohesin, primarily enriched at centromeres, supports cohesion until anaphase when proteolytic cleavage of a cohesin subunit triggers sister chromatid separation. We are interested in understanding exactly how these unloading processes of cohesin are regulated and how they are coordinated with other chromosomal events, such as condensin loading and maturation of mitotic centromeres. Our current effort is focused on a newly identified cohesin-interacting protein that appears to have a crucial role in these processes. Emerging lines of evidence suggest that the reorganization of chromosomes that occurs in prophase may be much more dynamic than anticipated before and that it may involve an active cross-talk between the arm and centromeric regions. We are currently testing this idea by using a combination of cell biology and RNA interference in human tissue culture cells. These studies will help us understand the high-

fidelity mechanism of chromosome segregation, defects of which lead to aneuploidy commonly associated with cancer and other human genetic disorders.

Dissection of the Mechanochemical Cycle of SMC Proteins

M. Hirano, T. Hirano

We have been using the *Bacillus subtilis* SMC protein (BsSMC) as a model system for studying the basic mechanism of action of SMC proteins. Like eukaryotic SMC proteins, BsSMC forms a V-shaped dimer in which a central hinge domain connects two long coiled-coil arms, each having an ATP-binding head domain at its distal end. During the past year, we have performed a comprehensive set of mutational analyses and have shown that the hinge domain has essential roles in modulating the mechanochemical cycle of SMC proteins. An initial interaction of the hinge domain with DNA leads to opening of the arms by triggering hydrolysis of ATP bound to the head domains, which are located approximately 50 nm away from the hinge. This conformational change allows the inner surface of the hinge domain to stably interact with DNA by an ATP-independent mechanism and primes ATP-driven engagement between the liberated head domains either within a dimer or between different dimers. Our results suggest that SMC proteins possess an intrinsic property to change their own conformations upon binding to DNA and initiate its manipulation in an energy-dependent manner. The basic principles of SMC action elucidated in this project will readily be applicable to our understanding of the actions of condensin and cohesin.

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EPITHELIAL CELL BIOLOGY AND CANCER

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Precancerous lesions of the breast are characterized by increased rates of proliferation and abnormal organization of epithelial cells, both properties routinely used by pathologists during prognostic evaluations. Over the years, the cancer research community has made tremendous strides to understand how oncogenic signals deregulate proliferation control and this has led to major discoveries that continue to shape cancer research. However, we do not know the mechanisms that regulate abnormal organization of epithelial cells in cancerous glandular structures. Although cell proliferation is common to both physiological and oncogenic stimuli, abnormal tissue/cell organization is restricted to a pathogenic state, suggesting that oncogenic signaling must possess unique mechanisms, not shared by physiological stimuli, that promote disruption of tissue/cell organization in precancerous lesions. Understanding the molecular mechanisms that regulate precancerous lesions will allow us to identify novel targets that will assist in diagnosis and treatment of early lesions.

Using mammary epithelial cells grown under three-dimensional culture conditions, we investigate the mechanisms by which epithelial organization regulates proliferation control under normal physiological conditions and how oncogenic signaling deregulates tissue organization and proliferation control.

MORPHOGENESIS OF EPITHELIA

The human mammary epithelial cell line, MCF-10A, when plated on the extracellular matrix, undergoes a program of proliferation and apoptosis to form proliferation-arrested three-dimensional acini-like structures that share several properties with acini in an adult breast. To have an unbiased analysis of how MCF-10A cells undergo proliferation arrest during morphogenesis, we performed microarray analyses of proliferating, proliferation-arrested cells grown under three-dimensional culture conditions. We identified a group of 119 genes that are up-regulated during proliferation arrest, suggesting that these genes have important roles during morphogenesis-regulated proliferation control. These analyses led to the identification of a protein tyrosine phosphatase, PTPRO. Down-regulating expression of PTPRO promotes formation of acini-like structures that require a significantly longer time to undergo proliferation arrest, suggesting that the regulation of tyrosine phosphorylation status by PTPRO has an important role in regulating proliferation control. We are very excited about this observation because several studies suggest that PTPRO functions as a tumor suppressor in lung cancers. In collaboration with the Tonks lab here at CSHL, we are investigating whether PTPRO functions as a tumor suppressor in breast cancers.

ERBB2-INDUCED DISRUPTION OF EPITHELIAL ORGANIZATION

Our studies—aimed at understanding how activation of the oncogenic receptor tyrosine kinase, ErbB2, disrupts three-dimensional organization of epithelial cells—have taken too many twists and turns and have finally come to a clear and exciting conclusion.

We and other investigators have demonstrated that oncogenic signaling in polarized epithelial cells promotes disruption of apical-basal polarity and aberrant organization of cell layers; however, the mechanism by which this happens is not known. To gain insight into the mechanisms by which activation of ErbB2 disrupts organized epithelia, we first determined the temporal order by which oncogenic signaling by ErbB2 affects cell polarity. Using proteins that are asymmetrically localized in polarized epithelia, we determined that oncogenic signaling induces loss of apical polarity and that such a loss is initiated at a region defined as the apical-basal border. The apical-basal border is home to cell-cell junction structures, such as tight junctions that serve as permeability and membrane diffusion barriers in glandular structures. Formation and maintenance of cell-cell junction complexes at the apical-basal border is directed by the Par polarity complex, a protein complex composed of Par3, Par6, atypical protein kinase C

(aPKC), and the small GTP-binding proteins, CDC42/Rac1. Because ErbB2 disruption of cell polarity initiates at the apical-basal border, we investigated whether oncogenic signaling regulates the Par polarity complex. These analyses revealed a novel link between oncogenic signaling by ErbB2 and regulation of the Par polarity complex, where, on the one hand, ErbB2 associates with Par6/aPKC and, on the other hand, ErbB2 inactivates the active Par polarity complex. The importance of this interaction was determined by expressing a mutant version of Par6 (Par6K19), which fails to bind aPKC and thus prevents ErbB2 from inactivating the Par complex. Cell expressing the Par6K19 prevented the ability of ErbB2 to induce large multiacinar structures but did not have a significant impact on the ability of ErbB2 to promote proliferation. Thus, we have identified a novel signaling pathway that uncouples the ability of an oncogene to disrupt epithelial organization from its ability to promote proliferation.

Along the course of this investigation, we stumbled on to an unexpected observation: overexpression of Par6 promotes epidermal growth factor (EGF)-independent proliferation of MCF-10A cells and promotes epithelial morphogenesis under suboptimal concentration of EGF. This has got our attention because it is possible that Par6 overexpression may have a significant impact for sensitizing cells to the growth factor requirement in vivo. To gain insight, we have generated a series of *par6* mutants and determined that the ability of Par6 to promote EGF-independent proliferation is mediated by the ability of Par6 to activate aPKC. We are pursuing these studies to investigate the mechanism by which Par6 promotes EGF-independent proliferation.

ERBB2-INDUCED DISRUPTION OF THREE-DIMENSIONAL ACINI

Activation of ErbB2 in proliferation-arrested three-dimensional structures reinitiated proliferation by increasing cyclin E/Cdk2 activity, but not cyclin D1/Cdk4 activity. The increase in Cdk2 activity was coordinated by up-regulating cyclin E and down-regulating $p27^{kip1}$. These observations are consistent with several studies that report a strong correlation between increased Cdk2 activity and ErbB2 overexpression in breast cancers. More importantly, overexpression of ErbB2 does not show a significant correlation with cyclin D1 expression or increased Cdk4 activity. Thus, we have defined a system that models a condition observed in ErbB2 overexpressing cancers in vivo. We are in the process of investigating how ErbB2 directly regulates the cyclin E/Cdk2 complex; such analyses will lead to novel insights into the mechanisms by which ErbB2 promotes deregulated proliferation in vivo.

Although coexpression of ErbB1 and ErbB2 is associated with poor patient prognosis, the mechanisms by which receptor heterodimerization regulates tumor progression are not clear, in part, due to lack of methods that allow controlled activation of specific receptor heterodimers in mammary epithelial cells. We have developed an approach to activate ErbB1-ErbB2 heterodimers without interference from endogenous ErbB receptors. Using such a method, we demonstrated that although both ErbB2 homodimers and ErbB1-ErbB2 heterodimers were equally potent in activating the Ras/MAPK pathway, the heterodimers were more potent in activating the PI3′kinase and phospholipase Cγl pathways than ErbB2 homodimers. We combined the dimerization system with a three-dimensional cell culture approach to demonstrate that although both ErbB2 homodimers and ErbB1-ErbB2 heterodimers induced disruption of three-dimensional acini-like structures, only heterodimers promoted invasion of cells through the extracellular matrix. The ability of heterodimers to induce invasion required the ErbB1 kinase activity and required activation of PI3′kinase, Ras/MAPK, and phospholipase Cγl signaling pathways. Thus, we have identified cell invasion as a heterodimer-specific biological outcome, and we suggest that coexpression of ErbB1 may critically regulate invasive progression of ErbB2-positive breast cancers.

In addition to providing insights into mechanisms by which ErbB receptors regulate transformation of organized epithelial cells, we have thus uncovered a novel arm of oncogenic signaling that critically regulates epithelial organization. Since loss of epithelial organization is observed in various pathological states, such as chronic inflammation, our findings have broad significance for diseases associated with glandular epithelia in vivo.

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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 that must get processed and transported to the cytoplasm. Although much biochemical information is available regarding many of the factors involved in gene expression, the spatial and temporal aspects of gene expression and the dynamics of the nuclear domains that the gene expression machinery occupies are less well understood. During the past year, we have focused a significant amount of our efforts on two main areas: (1) examining the mechanism by which epigenetic marks are transmitted to daughter cells through the cell cycle and (2) characterizing centromere positioning and dynamics in living *Arabidopsis* plants.

DYNAMICS OF EuHMTase1 DURING DNA REPLICATION

Methylation at lysine 9 (K9) of histone H3 is associated with both constitutive heterochromatin (trimethyl K9) and facultative heterochromatin (mono- and dimethyl K9). Among the H3K9-specific methyltransferases, EuHMTase1 and G9a target euchromatin, whereas SET-DB1, Suv39h1, and Suv39h2 are responsible for tri-methylation of H3K9 in constitutive heterochromatin. To elucidate how the epigenetic state of gene activity is transmitted to cellular progeny through the cell cycle, we studied the methylation of histone H3K9 during DNA replication. We investigated the localization and dynamics of EuHMTase1 and G9a, which are involved in the regulation of gene activity. To provide insight into the transmission of the pattern of dimethyl H3K9 at euchromatin, we first investigated the subcellular localization of EuHMTase1. On immunolabeling of HeLa cells with an antibody against EuHMTase1, we observed a fluorescent nuclear signal in all cells; however, approximately 20% of the cells in an asynchronous population exhibited different patterns of fluorescent foci. These foci colocalized with sites of BrdU incorporation (Fig. 1), indicating that they represented sites of DNA replication. We used live-cell microscopy to follow the dynamics of YFP-EuHMTase1 throughout the cell cycle. Interestingly, the association of EuHMTase1 with DNA replication sites was observed throughout S phase, from early DNA replication foci (enriched in active genes) to late replication foci (constitutive heterochromatin). Because G9a is generally associated in a heterodimeric complex with EuHMTase1, we also examined its localization in HeLa cells by coimmunolabeling with sites of BrdU incorporation. Similar to EuHMTase1, G9a was localized at sites of DNA replication throughout S phase, suggesting that G9a and EuHMTase1 are both present, most probably as a complex, at sites of DNA replication. After DNA replication was completed, EuHMTase1 showed a diffuse nuclear localization. To further study the association

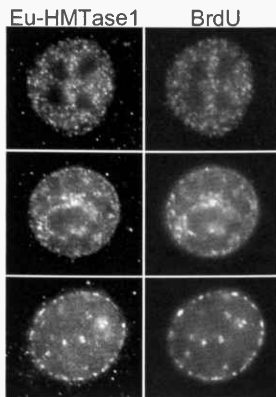

FIGURE 1 Immunolocalization in MCF-7 cells showing that EuHMTase1 colocalizes with sites of DNA replication throughout S phase. (Figure provided by Laurence Denis.)

and dynamics of EuHMTase1 with DNA replication foci, we performed a fluorescence recovery after photobleaching (FRAP) analysis using U2OS cells expressing YFP-EuHMTase1 protein. After bleaching a spot in the nucleus using a pulse with a 488-nm laser, we measured the recovery of fluorescence intensity in the bleached square. First, we analyzed the recovery after photobleaching YFP-EuHMTase1 at replication foci in early- or late-S phase by fitting exponential functions to the recovery curves. Single exponential functions could not be fitted to any of the recovery curves, indicating the presence of multiple distinct populations of YFP-EuHMTase1. However, by fitting bi-exponential functions, we could determine residence times of a rapidly exchanging fraction of YFP-EuHMTase1, which increased from 6.2 ± 1.5 s (mean ± S.D.) during early-S phase to 10.3 ± 5.8 s during late-S phase. The residence time of this fraction on non-S-phase chromatin was significantly shorter (3.1 ± 1.2 s; $p < 0.001$ when compared by *t*-test with early- or late-S phase). In addition, a fraction of approximately 40% bound with a longer residence time, increasing from 21.6 ± 8 s in non-S-phase cells to 106.4 ± 109 s in late-S-phase cells. The increased residence time at replication foci supports the idea of a functional association of Eu-HMTase1 at DNA replication sites. Interestingly, the residence time increased from early to later stages of S phase, which is likely a consequence of structural changes of chromatin at late replication foci. To determine the function of EuHMTase1 and G9a during DNA replication, we used RNA interference to deplete these proteins from p53-positive RKO cells. In cycling mammalian cells, EuHMTase1 depletion leads to a drastic diminution in the methylation (mono-, di-, and trimethylation) of lysine 9 at histone H3. In addition, we detected a significant increase of the G_0/G_1 population in EuHMTase1-depleted cells as compared to control cells, indicative of a cell cycle arrest in G_1. However, knockdown of G9a did not foster any obvious cell cycle modification. Under these conditions, BrdU incorporation was maintained, suggesting that EuHMTase1 is not directly involved in DNA replication per se.

We have identified a specific and dynamic association of the EuHMTase1/G9a histone methyltransferase complex with DNA replication sites. Because depletion of EuHMTase1 or G9a leads to a significant decrease of histone H3 methylation, these results establish the importance of DNA replication for the establishment and/or maintenance of the dimethylation pattern of H3K9. Moreover, they suggest that this complex has a central role in epigenetic maintenance of chromatin organization and gene expression. Although chromatin regions with different histone H3 methylation states globally replicate at different times during S phase, regions of facultative heterochromatin may be distributed throughout the genome and therefore may be replicated throughout S phase. This can explain the association of EuHMTase1 with replication sites throughout S phase. Furthermore, the EuHMTase1 complex may modify histone H3 with a dimethyl-K9 so as to mark it for incorporation into newly synthesized DNA irrespective of the chromatin state (euchromatin or heterochromatin). It will be of particular interest to decipher the kinetics of recruitment and activity of the EuHMTase1 complex relative to DNA synthesis and chromatin formation and the coordination of this event with further modifications of chromatin (i.e., H3.3 exchange at active sites of transcription, CENP-A loading at centromeres or H3K9 trimethylation at constitutive heterochromatin [catalyzed by SETDB1 or Suv39h1/ Suv39h2]).

CENTROMERE POSITIONING AND DYNAMICS IN LIVING *ARABIDOPSIS* PLANTS

The centromere, the primary constriction of the chromosome, is a DNA–protein structure that directs the movement of chromosomes during mitosis and meiosis. All centromeric regions contain specialized nucleosomes in which histone H3 is replaced by the centromere-specific histone H3 (CENH3). The organization and dynamics of the genome has been shown to influence gene expression in many organisms. Data from mammalian tissue culture cells have provided conflicting conclusions with regard to the extent to which chromatin organization is transmitted from mother to daughter nuclei. Here, we have taken advantage of *Arabidopsis thaliana*, with only 10 centromeres in diploid cells, to study the three-dimensional organization and dynamics of centromeres in interphase nuclei and through mitosis in living *Arabidopsis* plants. We developed transgenic *Arabidopsis* lines in which centromeres were tagged with a green fluorescent protein (GFP) fusion of the centromere-specific histone H3 (HTR12). Using four-dimensional live-cell imaging and quantitative analysis, we revealed that all centromeres localize predominantly at the nuclear periphery (Fig. 2) in different cell types in living *Arabidopsis* plants. In addition, we were able to demonstrate that *Arabidopsis* centromeres are constrained at the nuclear periphery during interphase, and the organization of endoreduplicated sister centromeres is cell-type depen-

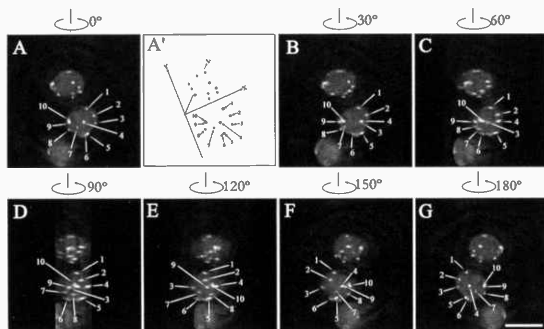

FIGURE 2 Maximum intensity projections of guard cells from the leaf of a living *Arabidopsis* transgenic plant. Projections at different angles showing centromeres labeled by HTR12-Venus in gray dots and chromatin labeled by histone HTB1-CFP. (*A*) Projection image of guard cells from 20 image sections with a z-interval of 0.2 μm. Centromeres in one guard cell were arbitrarily numbered 1–10. (*A'*) Diagram of positions of centromeres in *A*. The X axis represents the cell wall between two guard cells, the Y axis is vertical to the X axis, and the Z axis is directed to the bottom of the guard cell nuclei. Centromeres were defined as the brightest spots in the local fluorescent locus. Centromeres from sections 1–10 are represented by numbered arrows 1, 2, 4, 5, and 10. Centromeres from sections 11–20 are represented by numbered arrows 3, 6, 7, 8, and 9. (*B*) Projection image of guard cells after 30° counterclockwise rotation along the vertical axis. Centromeres (3, 6, 7, 9) at the bottom side of the nuclei move left. Centromere 9 can be observed at the periphery of the projected nuclei. Centromeres (4, 10) at the topside of the nuclei move right. (*C*) Projection image of guard cells after 60° counterclockwise rotation along the vertical axis. (*D*) Projection image of guard cells after 90° counterclockwise rotation along the vertical axis. Centromeres 4 and 7, which were at the center of the projected image at 0° (*A*), can be observed at the nuclear periphery of the projected nucleus. (*E*) Projection image of guard cells after 120° counterclockwise rotation along the vertical axis. (*F*) Projection image of guard cells after 150° counterclockwise rotation along the vertical axis. (*G*) Projection image of guard cells after 180° counterclockwise rotation along the vertical axis. Bar, 5 μm. (From Fang et al. 2005. *Mol. Biol. Cell 16*, 5710–5718.)

dent with predominant clustering in leaf epidermal cells and dispersion in root epidermal cells. The constrained movement suggests that centromeres anchor the chromosomes within the nucleus. Four-dimensional tracking of the entire set of centromeres through mitosis in growing root meristemic cells demonstrated that global centromere position is not precisely transmitted from the mother cell to daughter cells, indicating that precise positioning of centromeres/chromosomes is not essential for gene expression. However, positioning of subchromosomal domains and nuclear bodies may exist, resulting in distinct local environments that may affect the activities of individual genes or gene clusters. These results provide important insight into our understanding of chromatin organization and dynamics among different cells of a living organism.

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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 (melting), and 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.

ASSEMBLY OF A DOUBLE HEXAMERIC HELICASE

One of the factors that have severely hampered the study of viral initiator proteins such as E1 has been the inability to generate simple and robust assays for analysis of the complexes that these proteins form with DNA. This has led to the interesting paradox that the largest known complex, a double hexamer (dodecamer), is the best-characterized form of these proteins and virtually nothing is known about the complexes that precede formation of the double hexamer. We have made the development of an electrophoretic mobility-shift assay (EMSA) for E1 a very high priority. By using carefully controlled conditions and, in particular, by providing specific probes of precise length, we have been able to develop a robust EMSA that we have used to recapitulate the assembly of a double hexameric helicase on the viral origin of DNA replication.

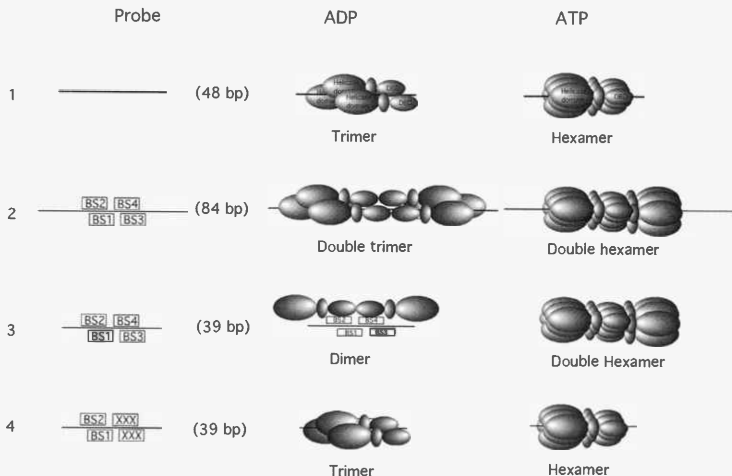

FIGURE 1 Probe dependence for E1 complex formation. E1 forms ADP-dependent trimers and ATP-dependent hexamers on probes without sequence dependence (*1*). In the presence of paired E1 BS, and on a longer probe, this translates into the formation of the functional forms of these complexes: a double trimer in the presence of ADP and a double hexamer in the presence of ATP (*2*). On a very short probe, in the presence of paired E1 BS, a dimer is formed in the presence of ADP and a double hexamer forms in the presence of ATP (*3*). If one pair of E1 BS is mutated (*4*), complex formation is identical to that observed for the nonspecific probe in *1*.

By using our newly developed EMSA, we could demonstrate that E1 has the intrinsic propensity to form trimers on DNA in the presence of ADP and hexamers in the presence of ATP. Formation of the trimer and the hexamer of E1 does not depend on the presence of particular DNA sequences. However, in the presence of the paired E1 binding site (BS) in the ori, this translates into formation of a head-to-head double trimer in the presence of ADP and a double hexamer in the presence of ATP (Fig. 1). An obvious possibility was that the ADP-dependent double trimer represented a precursor for the double hexamer. This would be consistent with a requirement for nucleotide binding for formation of the double trimer and for nucleotide hydrolysis for the formation of the double hexamer. By performing a time-course experiment, we could demonstrate that the double trimer indeed serves as a precursor for the double hexamer and that the double trimer is quantitatively converted into a double hexamer in the presence of ATP.

Our previous data has demonstrated that a second DNA-binding activity, in addition to the E1 DNA-binding domain (DBD), is present in the E1 helicase domain and that this activity is responsible for binding to the DNA sequences flanking the E1 BS. The lack of a requirement for E1 BS for trimer and hexamer formation indicated that formation of these complexes relied on this DNA-binding activity. Candidates for such an interaction are the residues that are present in the center of the ring structure, which these initiator proteins form. The structure of the helicase domains of initiator proteins from the papovavirus family, including SV40 T antigen and E1, contain a highly conserved structural element termed a β-hairpin which also has a counterpart in archaeal MCM proteins. This b-hairpin forms a narrow constriction on the inside of the ring lining the channel where DNA is predicted to be present. We generated a point mutation, H507A, in one of these conserved residues and tested it in a DNase footprint assay. As expected for a mutation that affects the DNA-binding activity present in the helicase domain, these mutations resulted in a protein that still bound to the E1 BS in a normal manner but that failed to bind to the DNA sequences flanking the E1

BS. This mutant also failed to form a trimer, indicating that the β-hairpin is directly involved in recognition of the flanking sequences and that formation of the trimer relies on the DNA-binding activity present in the helicase domain. H507A also failed to form a double trimer, and as expected from the precursor-product relationship between the double trimer and the double hexamer, H507A was also defective for double hexamer formation. Interestingly, we could show that this defect for double hexamer formation was directly caused by the failure to form a double trimer, since on a very short probe, where double trimer formation is not required, H507A formed a double hexamer.

These results indicated that on the ori-sized probe, the double trimer (DT) precursor in some way modifies the DNA structure to allow for the formation of a double hexamer. To determine whether this was the case, we performed permanganate reactivity assays in the presence of ADP under conditions where the double trimer, but not the double hexamer, forms. Under these conditions, we could detect significant reactivity, indicating that the double trimer is capable of melting double-stranded DNA and that likely the requirement for the double trimer precursor reflects a requirement for melting of the template prior to formation of the double hexamer. We could also demonstrate directly that the double trimer serves as a functional precursor for the complex that unwinds the DNA (i.e., the DNA helicase). Consequently, we can now identify the pathway by which the DNA template is prepared for initiation of DNA replication (Fig. 2). A dimer of E1 is responsible for initial binding to ori. Upon ATP binding, the double trimer forms and generates initial melting of the template. Upon ATP hydrolysis, the double trimer and the melted template serves as a substrate for formation of the double hexamer, which can unwind the template and generate single-stranded DNA. The ability to generate these different complexes and analyze them by EMSA now provides the opportunity to analyze initiation of DNA replication in unprecedented detail.

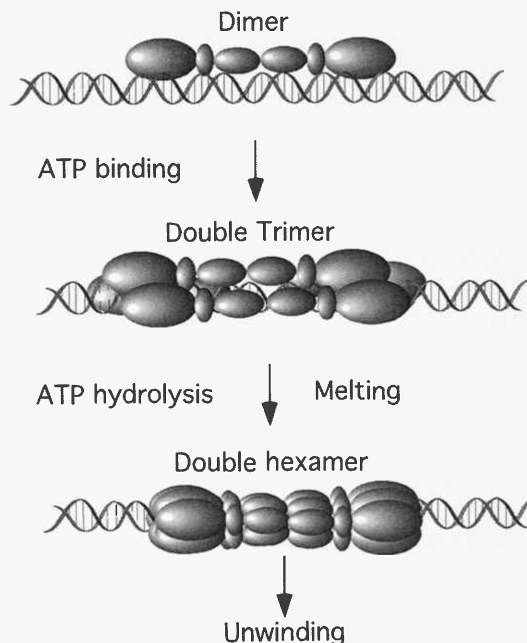

FIGURE 2 Assembly pathway for a functional E1 double hexamer. E1 binds to DNA as a dimer. In the presence of ATP, the dimer is converted to a double trimer, which melts the ori DNA. As a consequence of ATP hydrolysis, the double trimer is converted to a double hexamer, which unwinds the ori DNA generating free single-stranded DNA.

MUTATIONAL ANALYSIS OF THE E1 DBD SURFACE

The DBD of the E1 protein clearly is responsible for the site-specific DNA binding of which the E1 protein is capable. This site-specific DNA binding 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, on the basis of 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 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 63 surface residues for alanine, avoiding areas known from 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 16 mutants with defects in DNA replication. Four of these mutants were defective for expression in vivo, whereas the remaining 12 gave rise to wild-type levels of full-length protein. To determine which biochemical functions might be defective in these mutants, we expressed the 12 mutant E1 proteins in *Escherichia coli* and purified them. We tested these mutants in different in vitro assays related to DNA replication. From

the behavior of the mutants in these assays, we could group these mutants into four categories depending on which aspect of DNA replication was affected by the mutation. The results from in vitro DNA replication were particularly informative. Of the 12 mutants, 8 had severe defects for in vitro DNA replication. These mutants clearly had biochemical defects for replication-related processes. The remaining four mutants (Group I) still had activity for replication in vitro. These mutants are therefore likely to affect processes that are only required for DNA replication in vivo, such as regulation of viral DNA replication, for example, by cell cycle mechanisms.

Despite our efforts to avoid residues involved in DNA binding, four substitutions (Group II) had slight defects for DNA binding, most likely because these mutants affected the structure of the E1 DBD. Although these four mutants may also affect other activities, such effects would be obscured by the defect for DNA binding. A third group (III) consists of two mutants that have wild-type activity for all the biochemical activities that we can measure, with the exception of in vitro DNA replication. These two mutants are likely to affect a function required only for DNA synthesis, such as the interaction with cellular DNA replication factors. The final and fourth group consists of two mutants that have specific defects in the formation of the double trimer and double hexamer. These mutants also show the expected defects for unwinding activity.

It is apparent that there are many ways to disable the DNA replication activity of the E1 protein, which is a testimony to the multitude of diverse functions that this class of protein encodes. It is surprising, however, that so many of these activities appear to depend to some extent on the E1 DBD. This provides good evidence that the E1 DBD takes part in many biochemical activities that previously have been considered to reside in other domains.

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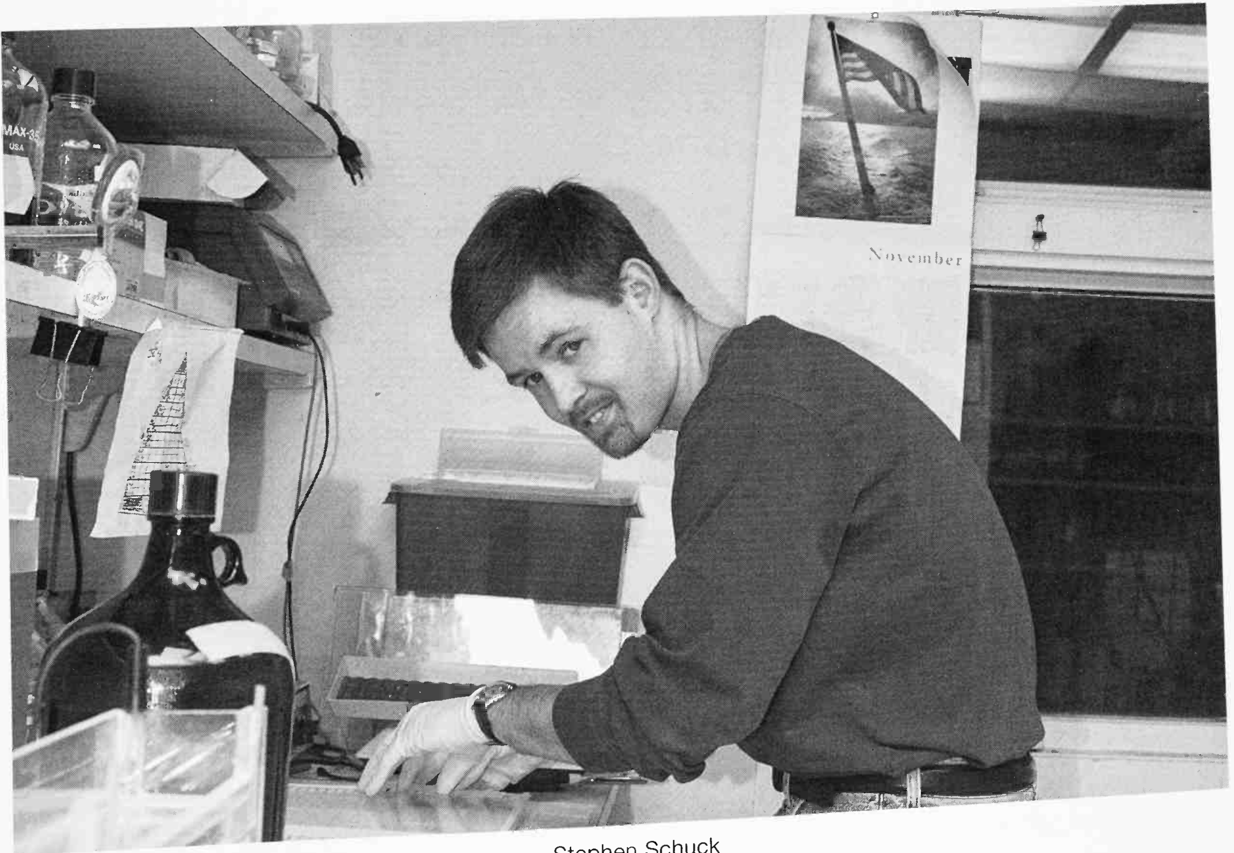

Stephen Schuck

DNA REPLICATION AND CHROMATIN INHERITANCE

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Chromosomal DNA replication in eukaryotes is a highly regulated process that involves the licensing of chromosomes at origins prior to S phase. The licensing occurs by the formation of a prereplicative complex (RC) at origins of DNA replication. The pre-RC consists of the origin recognition complex (ORC) that recruits Cdc6, Cdt1, and minichromosome maintenance (MCM) proteins to form a stable complex at each origin. Later, after activation of cyclin-dependent and Cdc7-Dbf4 protein kinases prior to entry into S phase, pre-RCs recruit other proteins that eventually promote the priming of DNA synthesis at each origin.

Previous studies from our laboratory have shown that ORC interacts with a regulatory protein called Cdc6 in an ATP-dependent manner and that this interaction changes how ORC interacts with the DNA. The addition of Cdc6 protein to the ORC-DNA complex causes a dramatic change in the way both ORC and Cdc6 bind to DNA. ORC normally binds to about 48 base pairs in the origin DNA and protects them from digestion in vitro by deoxyribonuclease I (DNase I). On addition of Cdc6, and only in the presence of ATP, the DNase I footprint is greatly extended to about 85 base pairs, resembling the region of the origin that binds proteins in vivo during the G_1 phase of the cell cycle, prior to the initiation of DNA replication. ATP hydrolysis by Cdc6 is required for formation of the extended footprint over the origin DNA since mutant versions of Cdc6 that lack ATPase activity cannot support the reaction. Furthermore, nonhydrolyzable analogs of ATP fail to support formation of the extended footprint, even though they do support Cdc6 binding to ORC on origin DNA.

Five of the ORC subunits are members of the AAA+ family of proteins. In collaboration with Huilin Li's group at the Brookhaven National Laboratory, we have begun to investigate the structure of ORC, the ORC-Cdc6 complex, and the structure of these complexes bound to DNA. Using computer-assisted reconstruction of single particles of ORC and ORC-Cdc6 complexes viewed by electron microscopy, the structure of these complexes has been determined. The technique provides a view of the structure of the proteins with a resolution of about 20 Å. ORC in the absence of any nucleotide forms an extended, three-domain structure about 18 nM long, sufficient to cover about 50 base pairs of DNA. In the presence of ATP or of ATPγS, an analog that is not easily hydrolyzed, ORC assumes a slightly different shape, but still with the same extended, three-domain configuration. Addition of Cdc6 to ORC in the presence ATPγS formed a ring structure with the same length dimensions as ORC. Thus, the extended footprint on origin DNA formed by ORC and Cdc6 most likely represents wrapping of the DNA around the protein complex. In the presence of ATP and in the absence of origin DNA, an ORC-Cdc6 complex is unstable.

To carry the structural information further, with Huilin Li's lab, we have developed a technique to extend the electron microscopy reconstruction data by tagging the subunits of ORC with an approximately 42-kD protein, the *Escherichia coli* maltose-binding protein. The amino termini and carboxyl termini of each of the ORC subunits were tagged, and the individual tagged proteins were assembled into the ORC complex. In this manner, most of the ORC subunits could be localized to the ORC structure. Electron microscopy reconstructions of ORC missing the smallest subunit, Orc6, enabled the location of this subunit to be determined.

The ATPase activities of the various complexes have been determined. As shown previously by other investigators, when ORC binds to the origin DNA, its intrinsic ATPase activity is reduced by about fivefold to sevenfold. Addition of Cdc6 greatly stimulates the ATPase activity of the complex, and the ATPase is dependent on both Cdc6 and ORC ATPase activities. In the absence of DNA, the ATPase activity of Cdc6 promotes dissociation of the ORC from the Cdc6. We have also found that Cdc6 also promotes the dissociation of ORC from non-origin DNAs, a reaction that requires the Cdc6 ATPase activity. This suggests that

ORC-Cdc6 complexes can only stably bind to authentic origin DNA sequences. Thus, Cdc6 contributes to the selection of origin DNA by ORC. We suggest that this mechanism exists in all species, particularly in those species such as *Schizosaccharomyces pombe, Drosophila*, and mammals where ORC by itself does not bind to specific DNA sequences. Cdc6 is structurally very similar to the largest ORC subunit Orc1 and it is likely that the ORC-Cdc6 complex, with its six AAA+ family members, is the real functional complex that determines origin specificity.

In collaboration with Rui-Ming Xu's lab here at CSHL, X-ray crystallography has been used to study the structure of subdomains of Orc1. These studies have identified the structure of a novel bromodomain-associated homology (BAH) domain in the amino terminus of Orc1 that interacts with a protein called Sir1. Sir1 contributes silencing of gene expression at two mating-type loci in yeast by binding to ORC, which is in turn bound to *cis*-acting silencer DNA sequences. We determined the structure of the Orc1 BAH domain bound to the ORC interacting region from Sir1 protein. The structure shows a multidomain interaction surface between Orc1 and Sir1 that allows the remainder of Sir1 to interact with other silencing regulators. In essence, ORC tethers Sir1 to regions of the genome that are destined to be heterochromatic. In mammalian cells, we have shown that ORC interacts with heterochromatin protein 1 (HP1), and we are in the process of determining how HP1 interacts with ORC and the consequences for heterochromatin formation in mammalian genomes.

We have also investigated the assembly of the human ORC complex. Unlike the yeast protein, human ORC does not form a stable complex throughout the cell cycle. The Orc1 subunit is degraded at the G_1- to S-phase transition, and Orc6 is not stably bound to the other subunits. We have demonstrated that human ORC is assembled in an ATP-dependent manner that is also dependent on the Orc4 and Orc5 ATP-binding motifs. Loading of Orc4 onto the Orc2 and Orc3 core complex requires prior binding of the Orc5 subunit. Once the Orc2,3,4,5 complex is assembled, Orc1 can bind, and then Orc6 interacts to form the entire ORC. However, the binding of Orc6 is regulated. We have previously demonstrated that Orc6 participates in other protein complexes in human cells at kinetochores and at the midbody during cytokinesis.

Current studies are aimed at identifying the function of the ORC subunits in human cells. In addition to specifying the starts sites for the initiation of DNA replication, ORC subunits are localized and have important roles at centromeres, kinetochores, and centrosomes, as well as heterochromatin.

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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 family of protein tyrosine phosphatases (PTPs), which, like the kinases, comprise both transmembrane, receptor-linked forms as well as nontransmembrane, cytoplasmic species and represent a major family of signaling enzymes. We are integrating a variety of experimental 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 the lab. Having completed her Ph.D., May Chen left for postdoctoral studies at Harvard. Bob Del Vecchio left to take up a position in industry, and Lifang Zhang is now pursuing a second postdoc. Ben Boivin joined us as a postdoctoral fellow having completed his Ph.D. in Bruce Allen's lab in Montreal. This was a challenging year—flooding in the Demerec building severely impacted tissue culture facilities, which slowed research considerably. Nevertheless, progress has been made in several areas.

FUNCTIONAL ANALYSIS OF THE PTP FAMILY

Unlike the protein kinases, which are derived from a common ancestor, the protein phosphatases have evolved in separate families that are structurally and mechanistically distinct. We have now cataloged the human PTP genes and more recently have extended our genomic sequence analysis to other organisms. The PTPs, which are defined by the active site signature motif $HC(X)_5R$, are divided into the classical, pTyr-specific phosphatases and the dual specificity phosphatases (DSPs). The 38 classical PTP genes encode receptor-like proteins, which have the potential to regulate signaling directly through ligand-controlled protein dephosphorylation, as well as nontransmembrane, cytoplasmic enzymes. There are about 65 genes encoding a heterogeneous group that are broadly described as DSPs. In general they share the same catalytic mechanism as the classical PTPs, but the construction of the DSP active site allows them to accommodate pSer/pThr residues as well as pTyr residues in proteins. Nonetheless, in terms of physiological function, the "DSPs" may actually show preference for either Tyr or Ser/Thr residues. Some even target nonprotein substrates such as inositol phospholipids and RNA. The DSPs include the VH1-like enzymes, which are related to the prototypic DSP VH1, a 20-kD protein that is a virulence factor of vaccinia virus; and the cdc25s, which are key regulators of the cell cycle and various phosphatidyl inositol phosphatases including the myotubularins. Overall, there are approximately 100 human PTP genes, compared to 90 human PTK genes, suggesting similar levels of complexity between the two families. However, the number of genes only illustrates the minimal level of complexity in the family, with additional diversity introduced through use of alternative promoters, alternative mRNA splicing, and posttranslational modification. This structural diversity is indicative of the functional importance of the PTPs in the control of cell signaling. It is now apparent that the PTPs have the capacity to function both positively and negatively in the regulation of signal transduction. Furthermore, the PTPs have the potential to display exquisite substrate, and functional, specificity in vivo. Therefore, the definition of the "PTP-ome" provides a foundation for detailed analyses of the structure, regulation, and

physiological function of this important family of signal transducing enzymes.

To facilitate research on the PTPs within the signal transduction community, we are making our analysis of the PTP family available as a Web-accessible resource at two locations: http://ptp.cshl.edu and a replica site at http://science.novonordisk.com/ptp. This resource contains our database of PTP accession numbers, multiple sequence alignments, phylogenetic trees, structure files, annotated molecular graphics files, chromosomal mapping data, analysis of exon structure, pseudogenes, and disease linkages. The PTP database can be searched by key words or by sequence similarity using our BLAST server, which also provides a tool for phylogenetic classification of anonymously submitted sequences based on PTP domain homology and neighboring joining trees. This is proving to be a popular resource, and we are continuing to update the site with the goal of maintaining it as the definitive source of information on the PTP family for the scientific community.

RNA interference is a powerful approach to conduct loss-of-function analyses as a method for examining the physiological function of particular proteins in mammalian cells. Having defined the composition of the PTP superfamily in humans, we have now designed and constructed a library of short hairpin RNA constructs that will allow us to examine systematically the function of individual PTPs in a variety of cell systems. We are using the pMLP retroviral expression vector constructed by Ross Dickins in Scott Lowe's lab here at CSHL, which includes a green fluorescent protein (GFP) marker and a puromycin selection cassette for generation of stable lines. Our first application of this library is currently ongoing as a collaboration with Senthil Muthuswamy here at CSHL. We are using his three-dimensional culture system to explore the effect of attenuating PTP expression on MCF-10A mammary epithelial cell differentiation, as a first step to defining roles for members of the PTP superfamily in breast cancer.

THE DUAL SPECIFICITY PHOSPHATASES

Many of the 43 DSPs we defined in our analysis of the human genome sequence are uncharacterized. Phylogenetic analyses divided these enzymes into three classes, revealing characteristics that were used to prioritize further studies. Currently, we are focusing on the following:

JSP-1

Multiple DSPs have been identified that have the ability to dephosphorylate and inactivate various MAP kinases, constituting a complex response network for attenuation of MAPK-dependent signals. We identified a novel DSP and observed, contrary to expectation, that it had the capacity to *activate* Jnk specifically, hence JSP-1 (Jnk stimulatory phosphatase 1). In addition, we observed enhanced phosphorylation of MKK-4, which phosphorylates and activates Jnk. Furthermore, expression of a dominant-negative mutant form of MKK-4 abolished the activation of Jnk by JSP-1, suggesting a site of action of the phosphatase upstream of MKK-4 in the Jnk signaling cascade. This study illustrates a new potential tier of control of the Jnk signaling pathway and a novel aspect of the role of protein phosphatases in the control of MAPK signaling. This raised the possibility that JSP-1 may offer a different perspective to the study of various inflammatory and proliferative disorders associated with aberrant Jnk signaling.

We have now generated JSP-1 knockout mice, which contain a targeted deletion at exon 3 resulting in the production of a truncated, inactive protein of approximately 20 amino acids. These mice reproduce at normal Mendelian ratios, display no obvious physical abnormalities, and have a life expectancy similar to their wild-type controls. Histopathological evaluation of aged (18–24 months) wild-type, and knockout mice revealed no gross abnormalities due to JSP-1 ablation in heart, kidney, liver, or brain. However, aged JSP-1 knockout mice display splenomegaly and splenic lymphoid hyperplasia; the cause of this is currently unknown but is under investigation.

We are characterizing two aspects of their phenotype—one of which implicates JSP-1 in the regulation of innate immunity and the other indicates that JSP-1 may have a role in the neurodegenerative processes that lead to Parkinson's disease. The backcrossing programs are now nearing completion, which will allow us to study the phenotypes on a consistent genetic background. We are currently breeding F10 generation heterozygotes in the C57/BL6 background and F8 with BALB/c. Thus far, we have observed that ablation of JSP-1 leads to attenuation of the inflammatory response to gram-negative bacterial endotoxin, and the knockout mice display defective production of (TNF-α) in response to lipopolysaccharide (LPS) administration, suggesting an important role in the innate immune system. We have now extended the observation to show that the heterozygotes display an intermediate response.

The mechanistic basis for this effect is currently under investigation. We have been trying to identify a suitable cell type from the mouse in which to investigate the signaling pathways responsible for the defective TNF-α response. The goal was to find a cell type that, in culture, would recapitulate the in vivo response (decreased LPS-induced production of TNF-α) and that could be cultured in sufficient numbers for biochemical analysis of signaling downstream from Toll receptors, such as TLR4, which mediate the effects of LPS. Splenocytes isolated from JSP-1 knockout mice secrete decreased TNF-α in response to LPS compared to wild type; however, the low numbers and short lifespan of these cells make it difficult to perform biochemical analysis of the signaling pathways responsible for this difference. Similar results were also obtained with bone-marrow-derived dendritic cells, but again this system does not yield sufficient cells for a comprehensive biochemical characterization. We did not observe a difference in LPS-induced TNF-α production between resident macrophages from the JSP-1 wild-type and knockout mice; however, in bone-marrow-derived macrophages from F7 C57/BL6 mice, we observed a reduction of ~50% in TNF-α levels produced from knockout compared to wild-type animals. These cells can be generated in sufficient numbers to perform signal characterization, and preliminary evidence from the use of phosphospecific antibodies to TAB1 indicates that JSP-1 may act at the level of the TAK1/TAB complex, downstream from TLR4. Experiments are under way to confirm this finding and to dissect further the impact on TAK1 activity, JNK activity, and NF-κB signaling.

In addition, preliminary results indicate that following treatment of JSP-1–/– mice with MPTP (1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine), the activation of Jnk is attenuated compared to wild-type controls. MPTP is a neurotoxin that replicates most of the neuropathological features of Parkinson's disease. We are currently investigating the mechanism by which JSP-1 regulates the Jnk pathway in this context and the extent to which manipulation of JSP-1 may influence neurodegeneration in Parkinson's disease. These studies are being performed with Serge Przedborski and his colleagues at Columbia University.

DUSP23

The gene for this DSP is located at chromosome 1q22.23, a region associated with amplifications, translocations, and other abnormalities in multiple cancers. Our phylogenetic analysis assigned this DSP to a class of phosphatases that could be considered the most ancient of the PTPs, due to their relationship to enzymes found in bacteria and archaea, perhaps indicative of a role in a fundamental cell function. Preliminary data suggest that this DSP may be amplified in certain breast tumors. Using RNA interference, we have shown that disruption of its expression inhibits proliferation and transition through the cell cycle. The mechanistic basis for these effects is under investigation.

MK-STYX

Several members of the PTP superfamily possess conserved domains with core features of a PTP but which lack residues that are critical for catalysis. We have been focusing on one such protein, termed MK-STYX, which is closely related to the MAP kinase phosphatases, for example, MKP-1. Sequence homology is observed throughout the proteins, including with the noncatalytic amino-terminal segment of MKP-1, which is known to serve a regulatory function. MK-STYX is catalytically inactive due to two substitutions in the signature motif (IFSTQGISRS, compared to VHCQAGISRS in MKP-1). We observed that mutations in MK-STYX to "restore" the active-site His and Cys residues that are known to be critical for activity in MKP-1 created a form of MK-STYX that displayed enzymatic activity. Furthermore, we observed that mutations in the signature motif that restored activity changed the pattern of associated proteins that were observed to coimmunoprecipitate with MK-STYX. This suggests that the proteins whose association with wild-type MK-STYX is disrupted by mutations in the signature motif may represent functional MK-STYX "substrates"/binding partners. Using a mass-spectrometry-based analysis of MK-STYX–binding proteins, we have identified Ras-*G*TPase-activating protein SH*3* domain-*b*inding *p*rotein (G3BP) as a candidate "substrate" of MK-STYX. G3BP interacts with RasGAP and is an endoribonuclease. Moreover, G3BP has been implicated in the regulation of Ras signaling and RNA stability. We are currently investigating the functional significance of this association.

PTEN

PTEN is the prototypic tumor suppressor phosphatase; more than 90% of glioblastomas lose alleles from chromosome 10q that invariably includes the PTEN

locus (10q23-24). In work from our laboratory and others, PTEN has been shown to dephosphorylate both proteins and inositol phospholipids; however, only the lipid phosphatase activity of PTEN has been clearly demonstrated to have a central role in its ability to function as a tumor suppressor. The significance of its protein phosphatase activity and the identity of bona fide protein substrates are not yet clearly understood.

We have been using two-photon laser scanning microscopy coupled with biolistic gene delivery into organotypic slices of the hippocampus as the basis for an in vivo assay of PTEN function. The organotypic slice cultures offer a unique physiological system that provides an easy and quantifiable readout of PTEN function. To date, we have focused primarily on determining the effects of expression of PTEN on morphology of rat hippocampus cells (pyramidal neurons). Although there was no effect on spine density, expression of PTEN led to a reduction in spine size, which can be quantified. We have been working with a tumor-derived mutation in PTEN, the G129E allele, which we had previously characterized as having impaired lipid phosphatase activity, but in which protein phosphatase activity is intact. We have observed that expression of this mutant produces the same reduction in spine size as observed with the wild-type protein. Although other studies have suggested functional significance for the protein phosphatase activity of PTEN, the organotypic brain slice preparation has provided us with the first evidence of a function controlled by this protein phosphatase activity in a living cell within a tissue.

We are currently testing the effects of wild-type and mutant forms of PTEN on PI3 kinase signaling in neurons. Furthermore, recent studies from several labs have focused attention on the potential importance of sites of phosphorylation at the carboxyl terminus of PTEN, particularly in the context of control of cell migration. It has also been proposed that activation of PI3K may promote phosphorylation and stabilization of PTEN. We have generated mutant forms of PTEN in which some of these potential sites of phosphorylation have been altered, and we are testing the effects of their expression on neuronal spine size. Overall, we are testing the potential functional importance of PTEN autodephosphorylation—its ability to recognize its own carboxy-terminal phosphorylation sites as substrates. This would reveal an interesting parallel with the situation for PI3K, which possesses the ability to both autophosphorylate and phosphorylate exogenous lipid substrates. Our study, which focuses on the capacity of PTEN to function as a protein phosphatase in vivo, may reveal novel insights into the physiological importance of PTEN and improve our understanding of how it functions as a tumor suppressor.

REGULATION OF PTP FUNCTION BY REVERSIBLE OXIDATION

As might be anticipated for a family of enzymes that have such critical roles in the regulation of cell signaling, the activity of PTPs is tightly controlled in vivo. Recently, the production of reactive oxygen species (ROS), such as hydrogen peroxide, and the resulting posttranslational modification of proteins by reversible oxidation has been implicated in the regulation of tyrosine-phosphorylation-dependent signaling pathways initiated by a wide array of stimuli, including growth factors, hormones, cytokines, and cellular stresses. This represents a novel tier of control of tyrosine-phosphorylation-dependent signaling and is a major emphasis of the research efforts in the lab. Attention was drawn to the PTPs as targets of ROS because the signature motif of this family, [I/V]HCxxGxxR[S/T], contains an invariant Cys residue, which, due to the unique environment of the PTP active site, is characterized by an extremely low pKα. The low pKα promotes the function of this Cys residue as a nucleophile in catalysis, but renders it highly susceptible to oxidation with concomitant abrogation of nucleophilic function and inhibition of PTP activity. Work from several labs has now established that multiple PTPs are transiently oxidized in response to various cellular stimuli. Oxidation of the active-site Cys in PTPs can produce either sulfenic (S-OH), sulfinic ($S\text{-}O_2H$), or sulfonic ($S\text{-}O_3H$) acid. For oxidation to represent a mechanism for reversible regulation of PTP function, it is essential that the active-site Cys is not oxidized further than sulphenic acid, since higher oxidation is usually an irreversible modification. In the classical PTPs, such as PTP1B, we now have a molecular explanation for how this is achieved. In previous years in collaboration with David Barford (ICR, London, United Kingdom), we demonstrated that oxidation of the nucleophilic cysteine to sulphenic acid is accompanied by conversion to a cyclic sulphenamide species, which induces profound conformational changes at the PTP active site that both disrupt the interaction with substrate and expose the oxidized cysteine to the environment of the cell. This serves the dual purpose of preventing irreversible oxidation to higher oxidized forms and facilitating the reduction of the sulphenamide to restore the active form of the PTP. We

have discovered a mutant form of PTP1B that adopts this oxidized conformation in a stable manner. Currently, we are using this mutant as an antigen in a phage display approach, which will enable us to screen a large repertoire of antibodies in a single library. Purified PTP1B mutant protein (PTP1B-OX) was used as an antigen to generate an immune response in chickens and first-strand cDNA was synthesized using the isolated mRNA from bone marrow and lymph nodes. We are now constructing single-chain Fv antibody fragments. Preliminary indications suggest that we have generated a large library, and screens for antibodies that recognize the oxidized conformation specifically are now under way. Such antibodies would have the potential to trap the oxidized form of PTP1B in a cellular context and may prove to be powerful tools to characterize further this important regulatory process.

Until now we have been using a modified in-gel PTP assay to examine reversible PTP oxidation in a cellular context. In this assay, cells are lysed under anaerobic conditions in the presence of an alkylating agent, which irreversibly inactivates any PTPs that are present in the reduced, active state. Those PTPs that are in an oxidized state are protected from alkylation. The lysate is then subjected to SDS-PAGE in a gel that is cast to contain a radioactively labeled substrate. The sample is subjected to electrophoresis, and then the proteins in the gel are taken through a denaturation/renaturation cycle. The renaturation step is conducted in the presence of reducing agent, so that any PTPs that were oxidized are now restored to the active, reduced state. They are able to dephosphorylate the substrate in their vicinity in the gel, and their presence is visualized by autoradiography as the appearance of a clear area of dephosphorylated substrate on the black background of radioactively labeled substrate. This has proven to be a powerful approach and has allowed us to characterize the oxidation of several different PTPs in a variety of signaling contexts, for example, the reversible oxidation of PTP1B and TC-PTP in response to insulin. Nevertheless, the approach is limited by the ability of the PTP family members to renature in the gel—not all PTPs are able to do so, in particular, the receptor PTPs are unable to renature in this way and so will be missed in this assay format. During the last year, we have been trying to develop alternative assays.

We have been optimizing a protocol to use a chemical label to tag the oxidized PTPs in a strategy similar to the modified in-gel phosphatase assay. After blocking the cysteine residues of the active PTPs with an alkylation agent and performing a buffer exchange, we reduce the PTPs that were oxidized in the original sample and then label them with an alkylating agent that is linked to a biotin group. Therefore, the PTPs that were originally oxidized can now be measured by an avidin detection system. Using this protocol, we are already able to detect PTP oxidation upon several treatments such as hydrogen peroxide and UV irradiation. We also found that this method is highly sensitive. In addition, we are collaborating with Zhong-Yin Zhang (Department of Biochemistry and Molecular Biology, Indiana University) to use some of the broad-specificity small-molecule PTP inhibitors he has developed in a similar approach. We are still optimizing this new technique, and this year, we hope to use it to study the stoichiometry and the specificity of PTP oxidation in a variety of signaling contexts.

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RAS AND RHO GTPASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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Ras and Rho family members have key roles in cellular activities controlling cell growth control, differentiation, and morphogenesis. 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 neurological disorders. Our ultimate goal is to understand how aberrations in Ras and Rho signaling components contribute to the development of these disease processes. Toward this end, my lab has continued to define the functions of selected GTPases, their regulators, and effectors, in models of cancer and neurological disorders. Below are highlighted the main projects that have been carried out during the past year.

ROLE OF DOK PROTEINS IN MITOGENIC AND ONCOGENIC SIGNALING

Dok-1 (also called $p62^{dok}$) was initially identified as a tyrosine-phosporylated 62-kD protein associated with Ras-GAP in Ph^+ chronic myeloid leukemia blasts and in v-Abl-transformed B cells. This protein was termed Dok (*d*ownstream *o*f *k*inases), because 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, Dok-1 and Dok-2 share in common the ability to bind to a negative regulator of Ras, Ras-GAP, suggesting that they may serve critical, but possibly redundant, functions. We described previously that Dok-1 acts as a negative regulator of growth-factor-induced cell proliferation and that Dok-1 inactivation in mice causes a significant shortening of the latency of the fatal myeloproliferative disease induced by $p210^{bcr\text{-}abl}$. Strikingly, in collaboration with Dr. Pandolfi's group (Memorial Sloan-Kettering Cancer Center), we also found that mice lacking both *Dok-1* and *Dok-2* spontaneously develop a chronic myelogenous leukemia (CML)-like myeloproliferative disease, likely resulting from increased cellular proliferation and reduced apoptosis.

Together, our data show that the Dok proteins have prominent roles as negative regulators of mitogenic and oncogenic signaling. However, how these proteins exert their negative effect on mitogenesis and oncogenic transformation has remained elusive. Using *Dok-1* knockout cells and *Dok-1* mutants deficient in binding to specific Dok-1-interacting proteins, we found that Dok-1 interferes with platelet-derived growth factor (PDGF)-stimulated c-*myc* induction and Ras/MAPK activation by tethering different signaling components to the cell membrane. Specifically, Dok-1 attenuates PDGF-elicited c-*myc* induction by recruiting Csk to active Src kinases, whereupon their activities and consequent c-*myc* induction are diminished. On the other hand, Dok-1 negatively regulates PDGF-induced MAPK activation by acting on Ras-GAP and at least one other Dok-1-interacting protein. Most importantly, we demonstrated that the actions of Dok-1 on both of these signaling pathways contribute to its inhibitory effect on mitogenesis. Our data suggest a mechanistic basis for the inhibitory effect of Dok-1 on growth-factor-induced mitogenesis and its role as a tumor suppressor.

ROLE OF RAP1 SIGNALING IN EPITHELIAL MIGRATION AND CELL-CELL ADHESION

The Rap1 protein, a member of the Ras family, was originally identified as an antagonist of oncogenic Ras, but more recent studies indicate that the function of Rap1 is largely independent of Ras. Rap1 is fundamental for the maintenance of cell-cell contacts between nascent epithelial cells and promotes cell movement. Importantly, a growing body of evidence suggests a dysregulation of Rap1 signaling in malignant processes. The signaling pathways mediating these effects of Rap1 remain largely unknown. We previously described the identification of the mammalian junctional protein AF-6 as a Rap1-interacting protein. To demonstrate the physiological relevance of this interaction, we decided to carry out functional analy-

ses of their orthologs (DRap1 and Canoe) in the more genetically tractable *Drosophila* system. We found that both DRap1 and Canoe are required for epithelial migration events in the embryo and that Canoe acts as a downstream effector of DRap1 in this process. Together, these experiments show that Canoe/AF-6 is a "bona fide" effector of Rap1 in the process of epithelial migration.

More recently, we identified a Drosophila Rap1-specific exchange factor, DPDZ-Gef, which is responsible for Rap1 activation in migrating embryonic epithelia. Genetic and biochemical experiments showed that the *DPDZ-Gef* gene is indispensable for epithelial migration in the embryo and that it activates the Rap1/Canoe pathway. Interestingly, the mammalian counterpart of the DPDZ-Gef protein has been described to interact with the adherens junctional protein, β-catenin and, like AF-6/Canoe, localizes to cell-cell junctions. Our data reveal a novel Rap1-dependent pathway key in regulating epithelial cell migration and adhesive processes that likely are conserved across species.

ROLE OF RAS AND RHO SIGNALING IN NEURONAL DEVELOPMENT

Accumulating data indicate 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. Moreover, aberrant Rho signaling has also been shown to underlie defects in axon regeneration. A major challenge has been to unravel how Ras and Rho signaling components affect neuronal development and function. Our studies in collaboration with Drs. Zhu, Malinow, and Svoboda here at CSHL have provided insights into how perturbations in Ras family members can impact neuronal function. Furthermore, we obtained data demonstrating a role for two regulators of the Rho GTPases, oligophrenin-1 and DOCK7, in dendritic spine morphogenesis and axon formation, respectively, of which a brief summary is given below.

Oligophrenin-1 (*OPHN1*) encodes a Rho-GTPase-activating protein (Rho-GAP), whose loss of function has been associated with nonsyndromic or nonspecific X-linked mental retardation (MRX). More recent studies have also reported the presence of OPHN1 mutations in families with mental retardation associated with epilepsy and/or cerebellar hypoplasia. Until recently, nothing was known about the function of OPHN1 in the brain. We obtained evidence for the requirement of OPHN1 in dendritic spine morphogenesis of hippocampal neurons. Using RNA interference and antisense approaches, we showed that knock down of OPHN1 levels in CA1 pyramidal neurons in hippocampal slices results in a significant decrease in dendritic spine length and that this spine length phenotype is mediated by the Rho/Rho kinase pathway. In addition, we recently demonstrated a biochemical interaction between OPHN1 and Homer, a postsynaptic adaptor molecule involved in spine morphogenesis and synaptic transmission. An interaction between OPHN1 and Homer raises the intriguing possibility that OPHN1 acts downstream from glutamatergic receptors to regulate RhoA activity in spines, and thus spine morphogenesis. Our future experiments are geared toward addressing these interactions and their functional significance in determining dendritic spine morphology, calcium signaling, and synaptic plasticity. Together, our studies will provide insight as to how *OPHN1* mutations compromise cognitive function.

DOCK7 is a novel Rac-interacting protein we recently identified in a search for regulators of Rac GTPase involved in the early steps of neuronal development. Blast searches revealed that DOCK7 belongs to the DOCK180-related superfamily of proteins, which emerged as novel activators of the Rho GTPases. We found that DOCK7 is highly expressed in major regions of the brain during early stages of development. Importantly, we observed that DOCK7 is asymmetrically distributed in unpolarized hippocampal neurons and becomes selectively localized to the axon as the neuron polarizes, suggesting a role for this protein in the early steps of axon formation, and hence in neuronal polarization. Using a culture of dissociated primary hippocampal neurons as a model system, we demonstrated that this is indeed the case. Knock down of DOCK7 expression prevents axon formation, whereas overexpression induces the formation of multiple axons. We further demonstrated that DOCK7 and Rac activation lead to phosphorylation and inactivation of the microtubule destabilizing protein stathmin/Op18 in the nascent axon and that this event is important for axon development. Our findings unveil a novel pathway linking the Rac activator DOCK7 to a microtubule regulatory protein and highlight the contribution of microtubule dynamics to axon development. Together, our studies contribute to defining the molecular mechanisms by which neurons acquire their polarity and may also shed light on axon regeneration processes.

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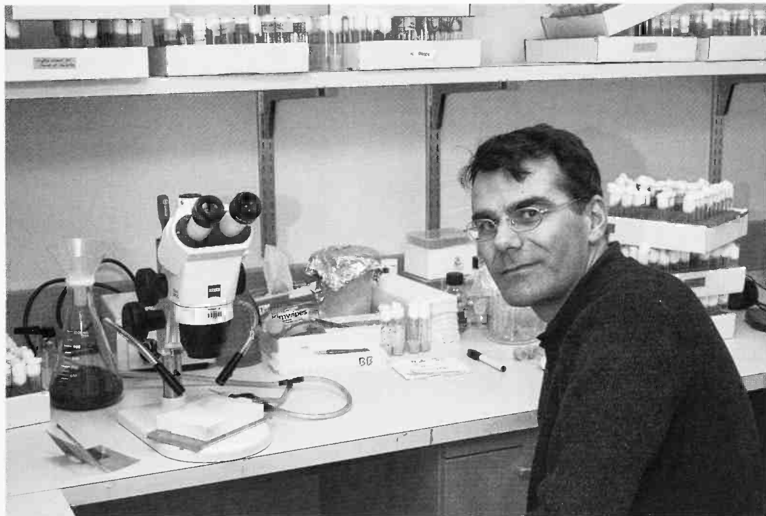

Benjamin Boettner

NEUROSCIENCE

CSHL neuroscientists are continuing to make extraordinary progress toward understanding many aspects of normal brain structure and function, as well as what goes wrong in several major diseases including Alzheimer's and Parkinson's diseases, schizophrenia, and autism.

Writers and poets know that memory is suffused with pain, ecstasy, fear, and desire. Science now supports the notion that intense emotions drive the changes in our brains that encode learning and memory. As part of his groundbreaking studies of the molecular basis of learning and memory, Robert Malinow (in collaboration with Anthony Zador) recently discovered that fear conditioning—a form of learning and memory—is encoded by the movement of proteins called AMPA (α-amino-3-hydroxy-5-methyl-3-isoazole) receptors into synapses in a portion of the brain known to be responsible for basic instinctual learned responses.

Karel Svoboda explores how information gathered from our senses is converted first into electrical activity and then transferred into memories through changes in synapses, neurons, and neural circuits. He combines a variety of powerful imaging techniques with rodent models to probe the brain's circuitry and how it changes over time. Many synaptic connections in the brain persist for months or years, but the protein components that form such connections between neurons are much shorter-lived. In addition to the work of Malinow, Svoboda's group recently helped solve this paradox by discovering a mechanism that controls the comings and goings of proteins at synapses.

When synaptic connections break down, memory is destroyed. Yi Zhong studies this breakdown, which underlies neurodegenerative disorders such as Alzheimer's disease. In his lab, researchers use the fruit fly brain as a model system for studying corresponding human genes that affect brain function in Alzheimer's patients. This analysis is revealing new clues about the disease and is providing insight into the general molecular mechanisms of learning and memory. Zhong's recent work has focused on neurofibromatosis 1 (*NF1*) and other genes implicated in Alzheimer's. His group has uncovered the pathways by which *NF1* controls learning and memory and continues to explore the mechanisms that underlie β-amyloid-induced neurodegeneration and memory loss in Alzheimer's disease.

Josh Huang is deciphering how the brain develops neural networks that respond to GABA (γ-amino-*n*-butyric acid), the primary inhibitory neurotransmitter in the brain. This year, Huang's group discovered that a cell adhesion protein called CHL1 has a major role in directing how neurons controlled by GABA connect to other neurons. These findings may have significant implications for understanding schizophrenia and other common neurodevelopmental and psychiatric disorders.

Even fruit flies need to learn and remember in order to survive. Moreover, by comparing the mechanisms of learning and memory in flies, humans, and other organisms, Tim Tully has discovered ancient, fundamental molecular pathways of memory that are shared from flies to man. Tully and his colleagues have uncovered some 170 candidate memory genes (CMGs) in fruit flies and are in the process of elucidating the roles of these genes in learning and memory.

Josh Dubnau is using several approaches to identify the molecular and anatomical pathways that underlie learning and memory in flies. His group has identified several genes that the researchers believe are involved in a mechanism that stores memories in response to external experiences. They are also using a genetic technique to map the neuroanatomical circuitry involved in memory processing in flies. Finally, Dubnau is pioneering an "artificial evolution" strategy with the goal of uncovering the network of gene interactions that govern learning and memory.

Partha Mitra is one of a number of CSHL neuroscientists who combine theoretical, computational, and experimental approaches to exploring brain function. His group is continuing to develop mathematical algorithms and powerful software necessary for making sense of large volumes of neurological data. In the experimental realm, Mitra is collaborating with researchers at City College New York to uncover new information about song learning in zebra finches, which is similar to speech acquisition in humans.

Carlos Brody is interested in how neurons interact with one another to form the neural networks that underlie rapid decision-making and short-term or "working" memory. His group is also exploring how time

and temporal patterns are sensed and represented in the brain. This year, Brody's group joined those of Zach Mainen and Anthony Zador to create a Center for the Neural Mechanisms of Cognition (CNMC). The goal of the Center is to develop rodent models that combine behavioral training with electrophysiological recordings to understand the neurological basis of several complex brain functions including learning, memory, time perception, motivation, and decision-making.

The ability to direct attention to one sound, for example, a particular person's voice, while ignoring other concurrent sounds is called "selective auditory attention" (a.k.a. the "Cocktail Party Problem"). Little is known about how the brain prioritizes sounds in this way. By recording the activity of brain neurons as rodents perform learned behaviors that require selective auditory attention, Zador and his colleagues have begun to reveal the neurological basis of a fundamental property of the brain.

Mainen studies how odors are detected, represented in the brain, and transformed into decision-making and other behaviors. A few years ago, his group discovered that rats (and probably sommeliers and other humans) can respond quickly and accurately to odors in a single sniff. Mainen and his colleagues continue to study the implications of this finding. From a "neuroeconomic" perspective, Mainen is studying how the brain deals with the biological "costs" of decision-making (e.g., uncertainty, effort, and delay). His group is also investigating the effects of psychoactive drugs in rodents to uncover neural circuits that might be targets of improved therapies for schizophrenia, Parkinson's disease, and other disorders.

Alexei Koulakov uses mathematical methods to explore how real-world neurons form functional networks in the brain. Some of his work has generated robust theoretical models of visual and olfactory neural circuits—models that match experimental observations and, importantly, make testable predictions that are likely to reveal new clues to brain structure and function.

One of the central questions of biology is how changes in gene activity transform one type of cell into other types. Cold Spring Harbor Fellow Lee Henry studies this process, called cellular differentiation, by using taste bud development as a model system. Taste buds comprise unspecialized "progenitor stem cells" at their periphery. These cells become specialized taste receptor cells within the center of the bud. Different taste receptor cells respond to different tastes (sweet, sour, salty, and bitter). Henry is working to define taste at the level of individual genes. To do this, he and his colleagues have developed a method that allows the gene products of individual cells to be attached to magnetic beads, which are then "read" using a series of chemical modifications. By comparing specialized taste receptors to unspecialized stem cells, the researchers aim to understand how individual taste receptor cells tell our brains, for example, "this is sweet."

The simple molecule nitric oxide (NO) has emerged as one of the key signals that controls neurogenesis, i.e., when and where stem cells in the brain become specialized neurons. Grigori Enikolopov uses fruit flies, frogs, and mice as models to study how NO controls neurogenesis in the human brain. His group focuses in part on exploring how new neurons are generated in the developing and adult brain under conditions of health and disease, and on the role that NO may have in the development of new nerve cells. These studies have led Enikolopov to groundbreaking discoveries concerning the role that neurogenesis defects have in depression and other mood disorders.

A complete picture of brain function must include an understanding of how the shape of individual neurons and their patterns of connections contribute to brain structure. Dmitri Chklovskii pursues this goal by building theoretical models based on engineering principles that include physical, electrochemical, and other "design constraints." In 2005, his group completed and validated reconstructions of rat brain circuits from the geometry of known neural shapes. In related work, Chklovskii used mathematics and other approaches to discover why the brain is segregated into the familiar "gray matter" and "white matter" and why brain microcircuits are connected the way they are.

Holly Cline studies how specific patterns of connections among brain neurons arise and how these patterns can change over time. By using time-lapse imaging of developing tadpoles, Cline's group is revealing the fundamental molecular and cellular mechanisms that are likely to underlie brain plasticity across species, including in humans.

COMPUTATIONAL SYSTEMS

C. Brody S. Chakraborty
S. Chow
E. Glushenkova
S. Lima
C. Machens
M. Nikichenko
S. Pai

Our lab is interested in how neurons interact with each other to form networks that underlie flexible cognitive acts, 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. Our approach to these questions is both experimental and computational. During 2005, the lab joined in efforts by Zachary Mainen and Anthony Zador's labs here at CSHL to create the "Center for the Neural Mechanisms of Cognition" (CNMC). Our goal is to develop rodent behavioral training, and electrophysiological recordings, to the same quantitative control and finesse as used in highly developed primate models, but with greatly increased throughput and experimental manipulability. Within the CNMC framework, Shraddha Pai has trained rats to discriminate between sounds of different durations and will be investigating the neurophysiological underpinnings of time perception. Susana Lima, a postdoctoral member of Anthony Zador's lab, has been working with us to train rats in a two-stimulus-interval discrimination task, previously used with much success in primates, and which will allow us to investigate short-term memory and decision-making in rodents. In computational efforts, Santanu Chakraborty completed his Ph.D. thesis on analysis and computational modeling of slow time covariations in persistent activity networks in goldfish. Christian Machens has extended his models of persistent activity mechanisms for short-term and working memory. Finally, Maxim Nikitchenko has continued the project started by previous lab member Amandine Penel, investigating the psychophysics of temporal pattern perception in humans.

Determining the Neuroanatomical Loci and Electrical Correlates of Temporal Bisection in the Rat

S. Pai

Interval timing (IT) is the ability to estimate time, and is used in decision-making in tasks where timing is important. For example, in foraging, the renewability of food sources must be timed to maximize food intake. Identifying the sources and electrophysiological correlates of IT have been challenging due to the lack of a controlled behavioral paradigm in a model organism amenable to lesion studies and electrophysiology. Discrimination of pure-tone durations using the two-alternative forced-choice (2AFC) paradigm in rats is a powerful yet simple model to study relative IT. We have developed a behavioral paradigm in which rats discriminate pure-tone durations that are longer than a standard interval, from those that are shorter (temporal bisection). The 2AFC paradigm is extremely well-controlled, allowing us to precisely measure the accuracy and extent of temporal bisection, using psychophysical analysis. We plan to lesion different auditory structures—specifically, the medial geniculate body of the thalamus, the auditory cortex, and auditory striatum—using a combination of reversible and permanent lesioning techniques to determine the brain regions that infer the ability to time auditory stimuli. These experiments will also be conducted in animals that have learned to discriminate relative pitches of stimuli, thus distinguishing structures specific to timing from those required for more common audition-based decision-making. We will then conduct multielectrode extracellular recordings in identified regions to determine the electrophysiological correlates of relative IT. In addition to localizing structures responsible for timing- and pitch-specific capabilities, this project will contribute to our understanding of the different auditory structures in audition-based decision making.

Two-stimulus-interval Discrimination Tasks in Rodents

S. Lima

Much work in our lab has been based on data collected by Ranulfo Romo's group in Mexico, recording from monkeys trained in a two-stimulus-interval discrimination task. In such tasks, a first stimulus (f1) is delivered

and then there is a delay, typically lasting several seconds. A second stimulus (f2) is then delivered, and the subject must compare the two stimuli and make a two-alternative, forced choice as to which of the two is the larger (f1 > f2? Yes or No). The task therefore requires short-term memory to remember f1 in the delay between f1 and f2, a comparison computation, and decision making. One of the goals of the CNMC is to bring such high-level tasks, whose neurophysiology is often explored with monkeys, into the rodent domain. This would provide a far more tractable model system for data collection and manipulation of brain activity. We have begun, and are focusing on, developing protocols to train rats in two-stimulus-interval discrimination tasks.

Temporal Dynamics of Short-term Memory

C. Machens

In a continuation of our research on working memory, we have now focused on the temporal dynamics of neural activities in the prefrontal cortex. Electrophysiological data from awake behaving monkeys, recorded by Ranulfo Romo (Universidad Nacional Autónoma de México), show that the activity of individual neurons correlates with a remembered sensory stimulus. Within a time scale of a few seconds, many of these neurons show systematic changes in their firing rates. Even though these temporal changes differ greatly between cells, we have managed to reduce the apparent complexity by decomposing the firing rate of each cell into a few generic components. Standard dimensionality reduction techniques (such as principal component analysis) have shown that three generic components suffice. During the presentation of the sensory stimulus, the stimulus information shifts from the first to the second component; after the presentation of the sensory stimulus, we observe a further shift from the second to the third component. We are now working on building a model that explains and replicates the observed temporal dynamics and integrates it with our previously developed mutual inhibition network.

An Analytical Framework for Line Attractors

C. Machens

Line attractors have been proposed as a conceptual foundation for persistent activity and short-term memory in many neural systems. However, the process of designing neural network models of line attractors based on realistic, nonlinear neurons has so far proved extremely complicated. As a spin-off to our previous work, we have developed a general analytical framework for the construction of line attractor networks. This framework is based on a simple geometrical principle: local translational invariance in the connectivity matrix of a neural network. As special cases, our framework includes the two types of line attractor networks that have been developed in the literature so far: (1) the bump attractor model based on the so-called "Mexican hat" network connectivity with short-range excitation and long-range inhibition and (2) monotonic line attractors based on a network connectivity matrix that can be written as the outer product of two vectors. This largely mathematical work seeks to further our understanding of line attractor design.

Human Psychophysics of Temporal Perception

M. Nikitchenko

Following work begun in our lab by Amandine Penel, who now holds a faculty position in Marseille, France, we are investigating the psychophysics of the perception of temporal patterns. We have developed two new reporting methods: After subjects hear an auditory temporal pattern, presented as a series of short sound blips ("pulses"), we ask them to reproduce the pattern by (1) voicing it and (2) tapping it on a touchpad. By examining systematic deformations and variability between the presented and the reproduced temporal patterns, we can determine biases and perceptual properties in how the sounds are perceived by the subjects. We developed an algorithm to precisely and automatically determine onset times of individual pulses in voiced patterns. We will use our new methods to investigate the perception of patterns more complex than those investigated previously, i.e., patterns involving four or more pulses.

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PRINCIPLES OF BRAIN DESIGN

D.B. Chklovskii B.L. Chen
Y. Mishchenko
Q. Wen

Understanding brain function requires unraveling synaptic connectivity in neuronal circuits. Therefore, the main goal of our laboratory now is to assemble the brain wiring diagram. We pursue this ambitious goal from several directions. First, we assemble a probabilistic description of neuronal circuits based on light microscopy data. Although light microscopy does not establish synaptic connectivity unequivocally, it is, currently, the only feasible approach to complex neuronal networks, such as the cortical column. Second, 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 the vertebrate nervous system. Third, we are using sampled physiological data to assemble a statistical description of functional connectivity. In addition, we are analyzing assembled wiring diagrams to make connections between anatomy and behavior. This analysis is difficult because the appropriate level of abstraction is not known. To make progress in this direction, we attempt to determine "engineering" principles that governed brain evolution. These principles, like the laws of conservation in physics, can narrow down the set of possible scenarios and help focus our efforts in the search for the models of brain function. In 2005, we made excellent progress on all of these research directions. Some of the highlights include understanding segregation of gray and white matter in mammalian neocortex, assembling the missing parts of the *Caenorhabditis elegans* wiring diagram from existing electron microscopy analysis, explaining the placement of neurons in *C. elegans* by using advanced methods from computer engineering, assembling the potential wiring diagram of a cortical column, and the identification of multineuron connectivity motifs in rat neocortex, proving that neuronal morphology features—such as branching axons and dendrites and spines—are necessary to wire up a cortical column with small conduction delays.

RECONSTRUCTION OF THE *C. ELEGANS* WIRING DIAGRAM FROM ELECTRON MICROSCOPY

Because synapses are submicron objects, they can be unequivocally detected only with electron microscopy (EM). However, describing synapses of even a single neuron requires reconstructing neuropil over several hundred microns. In principle, this range of scales can be covered by serial section EM. In practice, however, neuronal circuit reconstructions are rare because this technique is laborious and time-consuming. The most extensive reconstruction, that of the *C. elegans* nervous system, relied on approximately 10^4 serial sections and took about 10 years. Even this reconstruction was not completed—the exact synaptic connectivity of the ventral cord has never been published. We are finalizing the *C. elegans* reconstruction by using White's EM photographs and lab notebooks, as well as newly obtained EM photographs (in collaboration with D. Hall, AECOM). This reconstruction is all but complete and provides the first "proof of principle" connectivity matrix of the full nervous system.

HIGH-THROUGHPUT AUTOMATED EM

Reconstructions on a scale larger than *C. elegans* cannot be done manually and require high-throughput automated EM, which could be based on one of the following technologies: transmission EM, high-voltage EM tomography, and serial block-face scanning EM. With serious efforts to develop high-throughput EM currently under way in several laboratories, we expect that the leading candidate will emerge in the next couple of years. Meanwhile, we are developing automated reconstruction algorithms that will be necessary to handle gigantic data sets to be generated by high-throughput EM (in collaboration with A. Koulakov). A successful algorithm must produce a connectivity matrix of neuronal circuits such as the one for *C. elegans*. In addition, it must extract the shapes of neurons and the locations of individual

synapses. Although this project is extremely challenging, we believe that this is the right time to do it because of recent developments in digital image processing, continuing improvements in computer performance, and the existence of high-level programming environments such as MATLAB.

AUTOMATED ALIGNMENT OF SERIAL SECTIONS

The crucial first step for reconstructions using transmission EM and, to a lesser extent, for the other two technologies, is alignment of serial sections. The need for alignment arises from irregular placement of the sections on the grid and from their physical distortion, including stretching and shear. Traditionally, such alignment is performed manually and is rather time-consuming, especially if significant distortion is present. We developed an automatic algorithm for section alignment that overcomes distortion (including nonlinear). To assess the quality of alignment, we image the two consecutive sections in red and green channels. Currently, we perform alignment of real-life stacks of hundreds of serial EMs for our collaborators. If funding becomes available, we will build a Web site for automated reconstruction open to the neuroscience community.

SEGMENTATION OF EM IMAGES AND 3D ASSEMBLY

In our approach, we first segment images into cross-sections of individual neurons in 2D and then string them together in 3D. Traditionally, 2D segmentation is done by manually tracing contours of the objects of interest. Our algorithm performs segmentation automatically and for the whole volume at once. One of the challenges was to overcome a nonuniform contrast across the image. The next step of the reconstruction is to assemble 2D cross sections into 3D shapes of axons and dendrites. We have developed an automatic algorithm based on evaluating proximity and similarity of the 2D cross sections. To validate our algorithm, we compare the results of automatic reconstructions with those done manually by the experts on the same data set.

OPTIMAL INFORMATION STORAGE IN NOISY SYNAPSES

Experimental investigations have revealed that synapses possess interesting and, in some cases, unexpected properties. We propose a theoretical framework that accounts for four of these properties: typical central synapses are noisy; the distribution of synaptic weights among central synapses is wide; synaptic connectivity between neurons is sparse; and synaptic weights may vary in discrete steps. Our approach is based on maximizing information storage capacity of neural tissue under resource constraint. On the basis of previous experimental and theoretical work, we use volume as a limited resource and utilize the empirical relationship between volume and synaptic weight. Solutions of our constrained optimization problems not only are consistent with existing experimental measurements, but also make nontrivial predictions.

WHAT DETERMINES THE PLACEMENT OF NEURONS IN THE BODY?

We pursued the hypothesis that neurons minimize the cost of wiring between them. By using a method borrowed from computer engineering, we found the optimal layout for the given wiring diagram and compared it with the actual layout. Although most neurons follow wiring optimization predictions, some are displaced significantly. Interestingly, we find that outlier neurons have additional developmental functions (they pioneer axonal growth along the ventral cord), which may explain their misplacement. This finding illustrates how comparing theoretical predictions of the optimization approach with experimental data leads to the detection of discrepancies, which point to functional constraints that were not included in the original formulation.

WHAT IS THE ROLE OF AXONAL AND DENDRITIC ARBORS IN INFORMATION PROCESSING?

Since *C. elegans* neurons do not have elaborate arbors, we addressed this question in the context of cortical neurons. We find that wiring up of a highly interconnected network such as the cortical column is a difficult problem. The solution to such a problem in the allotted volume requires all of the major features of neuronal morphology, such as axon and spiny dendrites. Therefore, one needs not look further to find a reason for their existence. To understand branching and specific placement of synapses, we need to invoke "path-length cost," which increases with the distance between synapses and cell bodies. This cost arises due

to delay and attenuation in dendrites. Minimization of the path-length cost predicts the shape of axonal and dendritic branches consistent with experimental data. This work provides an explanation for the functional significance of neuronal shape.

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Beth Chen

BRAIN DEVELOPMENT AND PLASTICITY

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Sensory input into the brain is essential for organizing brain connectivity and circuit function during development and for modifying neuronal circuits as a result of learning in both the developing and mature nervous system. The goal of the research in our lab is to determine the cellular and molecular mechanisms that regulate the establishment and modification of brain connections. Nervous system dysfunction may arise from failure of these mechanisms to operate during development. We address this issue by examining the structural and functional development of the brain and spinal cord in tadpoles and zebra fish. These animals are transparent, which allows us to observe directly the development of the brain in living animals using time-lapse imaging methods. In addition, we assess neuronal function using electrophysiological assays of synaptic connectivity and synaptic plasticity. We combine these studies with gene-transfer methods that allow us to test the function of genes of interest in brain development. 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.

HOMER EXPRESSION IN THE *XENOPUS* TADPOLE NERVOUS SYSTEM

Homer proteins are integral components of the postsynaptic density and are thought to function in synaptogenesis and plasticity. In addition, overexpression of Homer in the developing *Xenopus* retinotectal system results in axonal pathfinding errors. This year, we found that *Xenopus* contains the *Homer1* gene, expressed as the isoform, *xhomer1b*, which is highly homologous to the mammalian *homer1b*. The mammalian *homer1* gene is expressed as three isoforms: the short form *homer1a* and the long forms *homer1b* and *homer1c*. In *Xenopus*, we cloned three very similar variants of *homer1b*, identified as *Xenopus homer1b*.1, *xhomer1b*.2, and *xhomer1b*.3. *xhomer1b*.2 and *xhomer1b*.3 display up to 98% homology with each other and 90% similarity to mammalian *homer1b*. Furthermore, we demonstrate that *Xenopus* also contains a short form of the Homer 1 protein, which is induced by kainic acid injection and is homologous to the mammalian Homer1a. *Xenopus* Homer1b expression was unaffected by neuronal activity levels but was developmentally regulated. Peak protein expression of both isoforms correlated with the period of synaptogenesis. Within the brain, the spatial and temporal distribution of both Homer isoforms was similar in the neuropil and cell body regions. Homer1 was detected in motor axons. Differential distribution of the two isoforms was apparent: Homer1b immunoreactivity was prominent at junctions between soma and the ventricular surface; in the retina, the Mueller radial glia were immunoreactive for Homer1, but not Homer1b, suggesting that the retinal glia contain only the Homer1a isoform. Homer1b expression in muscle was prominent throughout development and was aligned to the actin striations in skeletal muscle. The high level of conservation of the *xhomer1* gene and the protein expression in the developing nervous system suggest that Homer1 expression may be important for normal neuronal circuit development.

VISUAL EXPERIENCE REGULATES mGLuR-MEDIATED PLASTICITY OF AMPA-R SYNAPTIC TRANSMISSION BY HOMER1A INDUCTION

A fundamental adaptive feature of neurons within a functional network is their ability to respond to afferent activity by changing the strength of synaptic connections. At most excitatory synapses, changes in ionotropic α-amino-3-hydroxy-5-methyl-3-isoazole-receptor (AMPA-R) transmission underlie changes in synaptic strength, whereas the ligand and voltage-dependent *N*-methyl-D-aspartate receptors (NMDA-Rs) and metabotropic glutamate receptors (mGluRs) are thought to modulate AMPA-R synaptic plasticity and downstream signaling events. Activation of mGluRs results in bidirectional plasticity of AMPA-Rs, yet little is known about how mGluRs mediate such changes in AMPA-R synaptic transmission. We considered the possibility that the interaction of mGluRs with cytoplasmic scaffolding proteins may affect mGluR-dependent plastici-

ty. We investigated the potential role of the Homer family of scaffolding proteins in modulating mGluR-mediated plasticity of AMPA-Rs in the retinotectal system of *Xenopus* tadpoles.

Brief mGluR activation leads to plasticity of AMPA-R synaptic transmission. To test whether mGluR-mediated plasticity of AMPA-R transmission is influenced by recent neuronal activity, we manipulated visual activity in *Xenopus laevis* tadpoles in vivo. We compared mGluR-mediated plasticity of AMPA-R transmission in optic tectal cells of tadpoles with low levels of previous synaptic activity (overnight in the dark) to transmission in neurons from animals following 4 hours of constant visual stimulation. mGluR-mediated plasticity of AMPA transmission was significantly decreased in neurons with recent activity. We tested the role of the activity-regulated mGluR scaffolding protein, Homer1a, in modulating mGluR-mediated changes in AMPA-R transmission. We found that by changing the ratios of Homer 1a to Homer 1b in vivo, either by induction of endogenous Homer1a by visual activity or by ectopic expression of Homer1a or Homer1b, we could change the direction of mGluR-mediated plasticity. This is the first evidence that mGluR-mediated changes in AMPA transmission can be regulated by Homer proteins in response to physiologically relevant stimuli.

Previous experiments in the our lab demonstrated that a 4-hour period of visual stimulation increases the rate of tectal cell dendritic arbor growth, regulates tectal cell intrinsic excitability, and controls retinal axon arbor growth. Here, we report that experience-dependent changes in Homer1a expression in optic tectal neurons provide a means to homeostatically regulate mGluR-mediated plasticity of retinotectal AMPA-R transmission.

COORDINATED MOTOR NEURON AXON GROWTH AND NEUROMUSCULAR SYNAPTOGENESIS ARE PROMOTED BY CPG15 IN VIVO

The development of axon arbors involves a series of orchestrated events that take place after the axon reaches the target. These events include recognition of sites in the target where branching takes place, formation of branches and synapses, and refinement of the arbor structure through retraction and elimination of synapses and branches. We examined the possibility that motor axon innervation of the periphery could develop through a dynamic process of branch addition, maintenance, and retraction, concurrent with the formation of synaptic connections by collecting time-lapse images of motor neuron axons as they elaborated complex arbors and formed neuromuscular synapses.

We have used in vivo time-lapse two-photon imaging of single motor neuron axons labeled with green fluorescent protein (GFP) combined with labeling of presynaptic vesicle clusters and postsynaptic acetylcholine receptors in *X. laevis* tadpoles to determine the dynamic rearrangement of individual axon branches and synaptogenesis during motor axon arbor development. Control GFP-labeled axons are highly dynamic during the period when axon arbors are elaborating. Axon branches emerge from presynaptic sites, suggesting that mechanisms that govern synapse formation may also affect axon arbor elaboration. These data indicate that motor neuron axon elaboration and synaptogenesis are concurrent and iterative.

We tested the role of candidate plasticity gene 15 (CPG15, also known as Neuritin) in motor neuron axon arbor development and neuromuscular synaptogenesis. Previous work from our lab has shown that CPG15 is a highly conserved, extracellular, GPI-linked protein that promotes axonal and dendritic arbor growth as well as synapse maturation in the central nervous system (CNS). Furthermore, CPG15 protein is targeted to axons in the developing CNS. One of the earliest sites of *cpg15* expression is in the ventral spinal cord, suggesting that it may also have a role in the development of motor neuron axons and neuromuscular synaptic connections. To test this idea, we coexpressed CPG15, yellow fluorescent protein (YFP), and synaptophysin cyan fluorescent protein (CFP) in single motor neurons. We imaged the developing axons in vivo and found a startling increase in the elaboration of the motor neuron axon arbors and a significant increase in neuromuscular synapses. Further analysis demonstrated that CPG15 expression enhances the development of motor neuron axon arbors by promoting neuromuscular synaptogenesis and by increasing the addition of new axon branches at sites of synaptic contacts.

REGULATION OF RETINAL AXON ARBOR ELABORATION AND BRANCH DYNAMICS BY SYNAPTIC CONTACTS

Patterned neural activity and synaptic transmission guide the remodeling of axonal arbors in the developing CNS by regulating the addition, stabilization, and elimination of branches as arbors grow. In the retinotectal projection of frogs and fish, the dynamic

rearrangement of axonal branches actively refines and maintains the retinotopic map. Previous work in the our lab had demonstrated a critical role for activity-dependent Hebbian mechanisms in controlling axon branch retraction. We showed that the cellular mechanisms involved retrograde signaling downstream from activation of postsynaptic tectal NMDA receptors, which effectively function as correlation detectors. These and other studies, including our studies on motor neuron axon development, suggest a central role for synaptic connections in axon arbor remodeling and raise the intriguing possibility that as synapses mature and strengthen, they may serve double duty as structural sites for axon branch stabilization.

To examine the role of synaptogenesis and synaptic maturation in the structural development of axonal projections during the formation of the topographic retinotectal projection, we coexpressed cytosolic fluorescent protein (FP) and FP-tagged synaptophysin (SYP) in small numbers of retinal ganglion cells in living albino *X. laevis* tadpoles to reveal the distribution and dynamics of presynaptic sites within labeled retinotectal axons. Two-photon time-lapse observations followed by quantitative analysis of tagged SYP levels at individual synapses demonstrated the time course of synaptogenesis: Increases in presynaptic punctum intensity are detectible within minutes of punctum emergence and continue over many hours. Puncta lifetimes correlate with their intensities. Furthermore, we found that axon arbor dynamics are affected by synaptic contacts. Axon branches retract past faintly labeled puncta but are locally stabilized by mature synapses with intensely labeled SYP puncta. Visual stimulation for 4 hours enhanced the stability of the arbor at intense presynaptic puncta while concurrently inducing the retraction of exploratory branches with only faintly labeled sites or no synaptic sites.

DEPOLARIZING GABAergic CONDUCTANCES REGULATE THE BALANCE OF EXCITATION TO INHIBITION IN THE DEVELOPING RETINOTECTAL CIRCUIT IN VIVO

Neurotransmission during development regulates synaptic maturation in neural circuits, but the contribution of different neurotransmitter systems is unclear. We investigated the role of $GABA_A$-receptor-mediated Cl^- conductances in the development of synaptic responses in the *Xenopus* visual system. Intracellular Cl concentration ($[Cl^-]_i$) was found to be high in immature tectal neurons and then falls during a period of several weeks. GABAergic synapses are present at early stages of tectal development, and, when activated by optic nerve stimulation or visual stimuli, they induce sustained depolarizing Cl^- conductances that facilitate retinotectal transmission by NMDA-Rs. To test whether depolarizing GABAergic inputs cooperate with NMDA-Rs during activity-dependent maturation of glutamatergic synapses, we prematurely reduced $[Cl^-]_i$ in tectal neurons in vivo by expressing the Cl^- transporter KCC2. This blocked the normal developmental increase in AMPA-R-mediated retinotectal transmission and increased GABAergic synaptic input to tectal neurons. Therefore, depolarizing GABAergic transmission has a pivotal role in the maturation of excitatory transmission and controls the balance of excitation and inhibition in the developing retinotectal circuit.

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GENETICS OF MEMORY IN *DROSOPHILA*

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A. Blum E. Kockenmeister

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 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

A. Altick, J.Dubnau [in collaboration with T. Tully and N. Sinha, Cold Spring Harbor Laboratory]

In collaboration with the Tully lab, we have used a combination of behaviorally specific training protocols and expression profiling with DNA chips to identify transcriptional responses during memory consolidation (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). 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, we have confirmed differential expression for nearly 60 of these transcripts. This effort has identified candidate memory genes (CMGs), which serve as entry points for molecular genetic investigation of gene function in memory. These CMGs then become fodder for in vivo genetic manipulations to forge mechanistic connections between individual gene pathways and memory formation. 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*, which are known components of a cellular mRNA localization machinery in oocytes, and *pumilio*, which is a translational repressor protein. A large number of the known components of the mRNA localization machinery, as well as of the apparatus for regulating cytoplasmic polyadenylation-stimulated translational control, also are differentially expressed. Genetic reagents to manipulate these pathways are extant in *Drosophila*.

Functional Anatomy

A. Blum, S. Dennison, J. Dubnau [in collaboration with A.S. Chiang, Taiwan]

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-lived 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 repressor transgene. In contrast, LTM is normal in radish mutants but is disrupted in CREB repressor transgenic flies. These and other genetical and pharmacological experiments indicate the presence of at least five mechanistically distinct temporal phases of memory (short-term, middle-term, ARM, and LTM).

Although a neural structure called the mushroom bodies has a demonstrated role in early memory and in memory retrieval at several time points, very little else is known about the anatomical circuitry underlying each of the above memory phases.

To map the neural circuits required for memory formation, storage, and retrieval, we are using a temperature-sensitive dynamin transgene, which disrupts synaptic transmission reversibly and on the timescale of minutes. With this approach, we are now able to reversibly silence reproducibly specific groups of neurons in vivo. We already have demonstrated a role for synaptic transmission in mushroon body (MB) neurons during memory retrieval but, surprisingly, not during acquisition or storage of early memory (Dubnau et al., *Nature 411:* 476 [2001]). These data suggest that the synaptic plasticity underlying olfactory associative learning initially reside in MB dendrites and/or upstream of the MB and that the resulting alterations in synaptic strength modulate MB output during memory retrieval. But virtually nothing is known about the neural circuitry involved in the subsequent consolidation of short-term, middle-term, anesthesia-resistant, or CREB-dependent LTM phases. And nothing is known about the downstream circuitry of memory retrieval. We are using a panel of Gal4 enhancer drivers to focus expression of this dynamin transgene within specific subpopulations of neurons. Given the right Gal4 lines, this approach permits us to functionally map the circuit requirements for learning, memory retrieval, and each of the above temporal phases of memory.

To select Gal4 lines for Functional Anatomy investigations, we have used two approaches. First, Gal4 insertion mutations in genes involved in memory permit a first-pass evaluation of the green fluorescent protein (GFP) reporter expression pattern of biologically relevant genes. These then are used in combination with the dynamin mutant to reversibly silence synaptic transmission. This strategy already has given us an entry to study ARM. An enhancer-trap allele of *radish* (C133) (Chiang et al., *Curr. Biol. 14:* 263 [2004]) does not label mushroom bodies, which are the primary anatomical focus of olfactory memory research in *Drosophila*. Instead, C133-driven expression reveals a number novel neuronal types, several of which project into MB (Fig. 1).

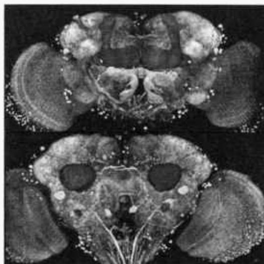

FIGURE 1 C133 reporter expression: A Gal4 insert in the *radish* gene drives GFP reporter expression in a complex pattern including a number of neurons that innervate the antennal lobes and MB. Frontal (*top*) and rear (*bottom*) views are shown.

Using the dynamin approach, we have shown a temporally graded requirement for neural activity within this population of neurons. In addition, we have a series of Gal4 lines that express in small subsets of these C133 neurons. One of these, which was initially identified by a Gal4 insertion mutation with a memory phenotype (Chiang et al., *Curr. Biol. 14:* 263 [2004]), drives expression specifically in APSP neurons (Fig. 2, left). This neuron type, which was first identified by C133 reporter expression, sends den-

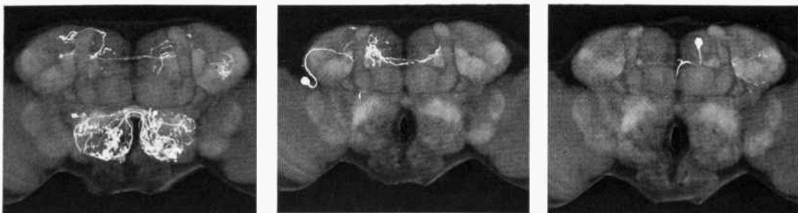

FIGURE 2 (*Left*) APSP neurons; (*middle*) DAL neurons; (*right*) DPM2 neurons.

drites into the antennal lobes. Axonal projections of these neurons terminate in the posterior superior protocerebrum (PSP). Using the dynamin approach, we have shown that activity in these APSP neurons is required after training for maintenance of an intermediate form of memory (not shown). In contrast, the APSP neurons are not required during learning (not shown). Our current efforts are focused on using several additional Gal4 drivers each of which express in specific neuron types that were identified in C133 (e.g., Fig. 2) to conduct an anatomical dissection of the neural circuits in which *radish* functions. In a similar manner, we also are making use of Gal4 insertions in a large panel of additional memory mutants (Dubnau et al., *Curr. Biol. 13:* 286 [2003]).

A second approach that we are using to select relevant Gal4 driver lines is based solely on their expression pattern, rather than their insertion in a "memory gene." In collaboration with A.S. Chiang, we have access to a growing panel of Gal4 enhancer lines that yield remarkably specific expression in neurons that send projections into different substructures of the MB. These identified MB "extrinsic" neurons constitute a significant fraction of the inputs, outputs, and modulation of MB. With the dynamin method, we can functionally manipulate these neuronal populations. The VAM neuron driver, for example, yields expression in four neurons whose dendrites project to the axon outputs of the mushroom body alpha lobe (Fig. 3). Axon outputs of the VAM neurons terminate in the PSP, nearby to the APSP neurons. With the dynamin approach, we have established a role for these neurons in an intermediate-term memory (Fig. 3). Like the APSP neurons, these VAM neurons appear to be dispensable for learning (not shown). Ongoing efforts are focused on filling in the rest of the circuitry for these "early" memory phases, as well as for long-term memory and for memory retrieval.

Selective Breeding of *rutabaga* Suppression

A. Altick, E. Kockenmeister, J. Dubnau

Genetic investigation of memory has revealed that underlying mechanisms are highly conserved across phyla. The cAMP cascade, for example, has been implicated in memory in both invertebrate and vertebrate animals, including humans. The informative power of genetics derives in part from identification of genes that influence phenotype, but also from analysis of gene interaction. Saturation mutagenesis for embryonic patterning, for instance, identified most of the relevant genes. Equally important, however, were second-site suppressing and enhancing screens. This approach allowed cell-signaling pathways and mechanistic insights to be distilled from what otherwise would be unconnected gene lists.

In the case of complex behavioral phenotypes such as memory, forward mutagenesis has identified a number of relevant genes (Margulies et al. 2005). Here too, the most informative instances are where mechanistic interactions among genes are understood. cAMP

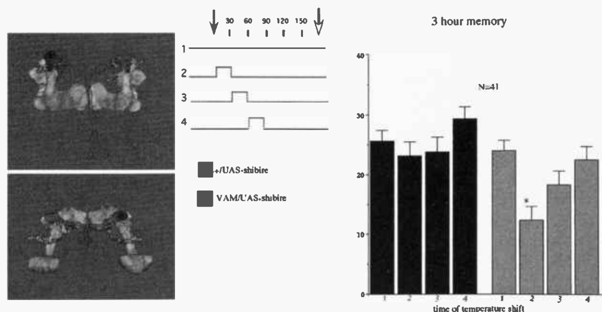

FIGURE 3 VAM neurons send dendrites to the mushroom body alpha lobes. VAM axon terminals terminate in the dorsal protocerebrum. VM neurons are required for an intermediate-term form of memory. Transient disruption of these neurons in the first 30 min after training (*2, middle panel*) or in the second 30-min period (*3, middle panel*) causes a partial disruption of memory measured 3 hr after training.

signaling is the most notable example. Assembly of genes into functional networks, such as the cAMP cascade, is the most challenging aspect of genetics. Although suppressor/enhancer screens have yielded some success, this approach is often not feasible for complex quantitative traits such as memory. Moreover, modifier screens are only designed to detect interactions between pairs of genes, but generally do not identify more complex gene networks. We are using experimental evolution with identified memory mutants (Dubnau et al., *Curr. Biol. 13:* 286 [2003]) in *Drosophila* as a strategy to screen for networks of gene interaction capable of suppressing or enhancing the role of cAMP signaling.

Selective breeding of extremes in behavioral phenotypes has a long and fruitful history. In contrast with screening for induced mutations, this strategy normally relies on the presence of preexisting (natural) genetic heterogeneity in a starting population. This method was first used to establish a genetic basis for several complex behavioral traits in a variety of species, including learning ability in *Drosophila*. This approach provided a means to estimate genetic variation in natural populations, to examine the process by which complex traits evolve through selection, and to investigate pleiotropy. Genetic analyses of the selected strains also demonstrated that phenotypic extremes typically derived from interactions among a constellation of alleles. Unlike forward mutagenesis, this quantitative genetic strategy relies on selection of combinations of alleles, thereby placing individual gene function within a network of interactions. This strategy also has potential to reveal mechanisms underlying evolution of phenotypic variation. In contrast with forward mutagenesis, however, quantitative genetic strategies on their own have not afforded an efficient means to identify and manipulate individual genes. Even with a variety of powerful quantitative-genetic mapping methods (e.g., QTL mapping), molecular identification of underlying loci has been challenging.

Each of the above genetic strategies thus offers complementary strengths for discovery of mechanisms underlying complex behavioral biology. Recent progress in genome-wide sequencing and expression profiling raises the possibility of combining the above methods. There is as yet no case, however, where artificial selection of molecularly identified alleles has been used for memory, no example where the evolution of a phenotype has been followed over successive generations at a mechanistic level, and no study where selection has been used to directly screen for suppression or enhancement of a known signaling pathway. We are using this strategy to identify networks of gene interactions. Our approach relies on artificial selection over the course of multiple generations to "evolve" combinations of known and molecularly tagged gene variants that interact to produce extreme levels of learning. Current efforts have focused on establishing the founding population for this selection experiment.

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STEM CELLS, SIGNAL TRANSDUCTION, AND DIFFERENTIATION

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Stem cells have a unique ability to self-renew and to produce progenitor cells that eventually generate differentiated cells. The cascade of transitions from stem cells to their differentiated progeny is under dynamic control, ensuring a rapid response to demands for more cells due to stress, damage, or altered environment. We study signals that regulate distinct steps in the differentiation cascade and guide cells to become integrated into the tissue they belong. We use fruit flies, frogs, and mice as models to study the regulation of division of stem and progenitor cells.

Our main focus is on how new neurons are generated in the developing and adult brain in health and disease. An important focus of our studies remains stem and progenitor cells in non-neuronal tissues. We also continue to generate new animal models to study neurogenesis. Finally, we started to focus on the role that neurogenesis may have in depression and other mood disorders.

NEURAL STEM AND PROGENITOR CELLS IN THE ADULT BRAIN

New neurons are constantly being generated in the adult brain (albeit in only a few areas) and can become fully functional several weeks after they are born. The functional role of this constant supply of new cells is still unclear; however, the fact that it takes place in the hippocampus, the area of the brain that has long been associated with learning and memory and, increasingly, with mood, has attracted much interest to the possibility that adult neurogenesis may be related to memory and mood disorders.

Recent findings indicate a close link between the action of a diverse range of antidepressant therapies and augmented generation of new neurons in the adult hippocampus. Moreover, these findings suggest that adult hippocampal neurogenesis not only accompanies, but is required for the behavioral effects of antidepressants of the selective serotonin reuptake inhibitors (SSRI) class (e.g., fluoxetine). This discovery may clarify several observations regarding the clinical course of action of antidepressant drugs. For instance, it may explain why an appreciable clinical effect of the drugs usually takes at least 3–4 weeks: This period is comparable with the time required for a detectable increase in neurogenesis in rodents after behavioral stimulation and may reflect the time required for neural stem cells to proceed through the neuronal differentiated cascade and become fully differentiated neurons.

One of the problems in understanding how antidepressants (or most of the other neurogenic stimuli) increase adult neurogenesis is that it is not known which steps of the neuronal differentiation cascade they affect. Particular targets (e.g., stem cells vs. early progenitors vs. advanced neuroblasts) may imply different molecular mechanisms of controlling cell division and survival, different circuits affected by the drugs, and different insights on the behavioral action of the drugs.

To address this problem, we generated a novel reporter mouse line (nestin-CFPnuc mice) that allows us to quantitatively assess changes in stem/progenitor cells of the adult brain. In this line, the reporter (cyan fluorescent protein) is fused to a nuclear localization signal, and the distribution of stem and progenitor cells in the neurogenic areas is visualized as a dotted pattern; this greatly reduces the complexity of the pattern and permits unambiguous enumeration of stem and progenitor cells.

We used this reporter line to define discrete steps in the neuronal differentiation cascade (leading from stem cells to differentiated granule neurons), based on the morphology of the cells, the marker proteins that they express, and their mitotic activity, thus generating the most complete current scheme of the differentiation cascade in the adult hippocampus.

We then used the reporter line and the elucidated scheme of the differentiation cascade to determine the target of the action of fluoxetine in the adult hippocampus. We found that fluoxetine does not affect division of stem-like cells in the dentate gyrus, but

increases symmetric divisions of an early progenitor cell class. We further demonstrated that this is the sole class of neuronal progenitors targeted by fluoxetine in the adult brain and that the fluoxetine-induced increase in new neurons arises as a result of the expansion of this cell class.

These results thus identify a specific functional target of fluoxetine, link early progenitor cell classes to the action of SSRI antidepressants, and, importantly, suggest a general strategy to investigate the changes induced by other neurogenic stimuli. We are now applying this approach to identify the targets of antidepressant drugs and treatments in the adult and developing brain.

STEM CELLS IN NON-NEURAL TISSUES

Tissue maintenance in the adult organism requires a constant supply of new cells to replace differentiated cells lost to stress and damage or destroyed as part of the normal cell death program. This replacement is made possible through the activity of adult tissue-specific stem cells. These cells are set aside during development or soon after birth, persist in the adult tissue, and produce committed progeny that eventually gives rise to differentiated cells. Self-renewal of adult stem cells and their production of lineage-committed progeny are controlled by a host of intrinsic and extrinsic factors and are primarily governed by the interactions between the stem cells and their microenvironment, the stem cell niche.

We found that in our reporter mouse lines (which were generated primarily to study neural stem cells), expression of the reporter (nestin-GFP or nestin-CFPnuc) marks stem and progenitor cells in several non-neural tissues. We have shown that such reporter-expressing cells can be found in the bulge region of the hair follicle where they represent a population of cells with stem properties and a capacity to generate neuronal cells in vivo and in vitro, in testis, where they persist as precursors to the steroidogenic Leydig cells, in the ciliary margin of the eye, and in the anterior pituitary. Furthermore, in the liver, reporter expression marks oval cells, which have long been purported to serve as stem cells after exposure to carcinogens or in the settings where the proliferative response of hepatocytes (a usual mode of liver regeneration) is suppressed. We now study the ability of these tissue-specific stem cells to demonstrate their properties in vivo, e.g., upon transplantation, or to be recruited to noncognate tissues for regeneration.

NO AND EARLY DEVELOPMENT OF THE *XENOPUS* NERVOUS SYSTEM

During the early steps of development, two major morphogenetic processes, cell division and cell movement, are tightly linked. Active cell duplication is required to generate a sufficient number of cells; however, it must be coordinated with precise morphogenetic cell movements during gastrulation and organogenesis to build a correctly structured organ or a tissue. In many cases, temporary cessation of cell division is an integral part of the morphogenetic program during early development. However, the nature of the signals that coordinate cell division and cell movement during development has been elusive. We investigated whether nitric oxide (NO), a signaling molecule with both antiproliferative and pro-motility potential, can link these two major morphogenetic processes. We used gain- and loss-of-function approaches to demonstrate that NO, affecting both the formation of cGMP and nitrosylation of key proteins, acts through RhoA to regulate cell proliferation and morphogenetic cell movements. Excess NO decreases proliferation but does not interfere with cell movement; in contrast, deficiency of NO increases proliferation and disturbs cell movements (manifested as defects in the neural tube closure and axis extension). We found that NO controls cell proliferation by regulating, in a cGMP-dependent way, the activity of Rho-dependent kinase (ROCK), a downstream target of RhoA, and that this regulation is essential for the distribution of the *cdk* inhibitor p21 between the nucleus and the cytoplasm and for its stability. We also found that NO regulates cell movement through nitrosylation of RhoA and through direct interactions with Dsh, a crucial regulator of RhoA in the planar cell polarity pathway. This affects cell polarity, which is essential for the directional morphogenetic movements of cells of the embryonic nervous system and the notochord. This concurrent NO-mediated control of convergent extension and cell division helps ensure that the crucial processes of cell proliferation and morphogenetic movements are coordinated during early development of the nervous system.

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June Hee Park

CONSTRUCTION AND PLASTICITY OF GABAERGIC CIRCUITS

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Brain functions that control behavior emerge from activities of vast underlying neural networks. In many areas of the vertebrate brain, neural networks often consist of repeated functional modules. The neocortex, for example, seems to have arisen from duplication of stereotyped local circuits with subtle specializations in different cortical areas and species. Unraveling the logic of the basic design and operation of local neuronal circuits is essential to the theory of brain function. The major components of neocortical local circuits are pyramidal neurons, which have relatively stereotyped anatomical, physiological, and molecular properties; the shear number and intricate design of these excitatory glutamatergic neurons endow them with enormous potential for information coding, storage, and plasticity. On the other hand, GABAergic interneurons, although a numerical minority, provide the largest degree of heterogeneity in the neocortex. Different classes of interneurons display highly distinct morphologies, physiological properties, connectivity patterns, and gene expression profiles. This rich diversity suggests that these inhibitory components have evolved not simply to balance excitation, but, through the variety and fine details of inhibition, to control neural communication and circuit configuration.

Decades of studies begin to reveal a major theme on the function of GABAergic interneurons: timing in neurons and networks. GABAergic transmission powerfully controls synaptic integration, probability, and timing of action potential generation. Interneurons also generate and maintain network oscillations and are thought to provide the temporal structures for coordinating the activity of neuronal populations and for orchestrating the dynamic formation of functional ensembles. The biophysical properties of at least certain classes of interneurons seem to be optimized to achieve physiological timing, such as fast, precise, and reliable conversion of excitatory inputs to powerful inhibitory outputs. Different classes of interneurons further display highly distinct axon arbors and innervation patterns, which distribute their outputs within and between cortical columns, among local neuronal populations, and among subcellular compartments of target neurons. These connectivity patterns therefore translate temporal regulation to discrete spatial domains and help group or segregate cell assemblies during all aspects of circuit operation. Unraveling the design, construction, and modes of operation of GABAergic circuits is therefore a key to understanding the architecture and computation of local neural circuits. My laboratory is studying the construction, plasticity, and function of GABAergic circuits in mice, using the neocortex and cerebellum as complementary experimental systems. We have focused on three areas in the past year.

SUBCELLULAR ORGANIZATION OF GABAERGIC SYNAPSES: L1CAMS AND ANKYRIN MEMBRANE SKELETONS

A striking feature of GABAergic innervation is the targeting of different classes of synapses to subcellular compartments of principal neurons (spines, dendrites, soma, and axon initial segment [AIS]). Subcellular spatial segregation of inhibitory synapses is essential for the precise regulation of input integration, spike probability, timing, and back propagation, but the underlying mechanism is unknown. Combining cell-type-specific transcriptional promoters, bacterial artificial chromosome (BAC) transgenic reporter mice, and high-resolution imaging, we have established powerful in vivo and in vitro systems to visualize and manipulate defined classes of GABAergic interneurons. Our study is beginning to outline the mechanisms underlying subcellular organization of GABAergic synapses in mammalian brain. First, we discovered that subcellular targeting of GABAergic synapses in the sensory 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 began to define the molecular signals directing synapse targeting in cerebellum. We discov-

ered that the ankyrinG-based membrane cytoskeleton is the key molecular machinery which, through subcellular recruitment of neurofascin, a member of the L1 family immunoglobulin cell adhesion molecules (L1CAMs), directs GABAergic innervation to the AIS. Third, we recently discovered that another member of L1CAM, CHL1 (close homolog of L1), is involved in targeting a different class of GABAergic synapses to Purkinje dendrites (F. Ango et al., in prep.). Both the ankyrin and L1CAM families contain multiple members localized to distinct subcellular compartments. Our general hypothesis is that members of L1CAMs recruited to subcellular domains by different ankyrins may constitute a set of "compartmental codes" in principal neurons for subcellular organization of GABAergic synapses. A major revelation is that subcellular organization of GABAergic synapses in superimposed upon an elaborate subcellular organization of ion channels. Such spatial alignment of biophysical properties and synaptic inputs upon the same macromolecular complex may confer the ultimate temporal precision for regulating electrical signaling within and among neurons. These studies suggest an unexpected link from subcellular molecular organization to synaptic connectivity and network properties. Our studies raise many more questions: What are the mechanisms of GABAergic synapse targeting in a more complex circuit such as the neocortex? What are the receptors in different classes of GABAergic neurons for L1CAMs?

ACTIVITY-DEPENDENT DEVELOPMENT AND PLASTICITY OF GABAERGIC SYNAPSES AND INNERVATION PATTERN: A NOVEL FUNCTION OF GABA

Another highly characteristic feature of the GABAergic innervation pattern is its local exuberance: A single basket interneuron axon in the rodent neocortex innervates hundreds of neurons in its vicinity and forms multiple, clustered synapses onto the soma and proximal dendrites of each target. Such exuberant innervation is likely crucial for effective control of spike timing and synchrony among target neurons, but the underlying mechanisms are unknown. We found that the maturation of perisomatic innervation is a protracted process influenced by sensory experience during adolescence. In addition, the basic features of perisomatic innervation can develop to a substantial extent in organotypic culture and are regulated by neuronal activity.

As direct mediators of neural activity, neurotransmitters are particularly well suited to sculpt synaptic growth and refinement in response to functional transmission. Indeed, signaling through glutamate regulates nearly all aspects of excitatory synapse development and lies at the heart of the emerging rules underlying activity-dependent plasticity. The role of GABA in the development of inhibitory synapses has not been explored due to technical and conceptual roadblocks. Using conditional knockout of GABA synthetic enzymes (GAD67) restricted to basket interneurons (Fig. 1), we recently discovered that the level of GABA synthesis and signaling are crucial in regulating interneuron axon growth and synapse formation in adolescent cortex, when GABA transmission has become largely hyperpolarizing. These results reveal a novel function of GABA in regulating inhibitory synapse and circuit development, which is distinct from its early trophic role largely due to depolarizing action, as well as from its classic role as an inhibitory transmitter. Because GAD67 level is strongly coupled to neuronal input, activity-dependent GABA synthesis and signaling may provide a cell-wide and synaptic mechanism to sculpt the innervation pattern of GABAergic interneurons. These findings beg a major revision in the current concept of GABA function in the vertebrate brain and raise a whole series of questions regarding the underlying mechanisms: What are the cellular and signaling mechanisms mediating GABA-regulated synapse and axon growth? What are the physiological mechanisms and function of activity-dependent regulation of GAD67 expression and GABA synthesis? Our findings suggest an unexpected parallel between the role of glutamate in regulating the morphogenesis of excitatory synapses and that of GABA for inhibitory synapses. On the other hand, our findings also imply fundamental differences between these two systems. For example, activity-dependent cell-wide regulation of "GABA resource" implies a novel logic for the plasticity of GABAergic synapses and innervation patterns.

MATURATION OF GABAERGIC TRANSMISSION AND CRITICAL PERIOD PLASTICITY IN VISUAL CORTEX

Maturation of GABAergic inhibition has been implicated in the onset of the critical period for ocular dominance (OD) plasticity, but the underlying cellular mechanism remains elusive. In particular, it is unclear whether GABAergic transmission regulates synaptic competition among converging inputs, a defining fea-

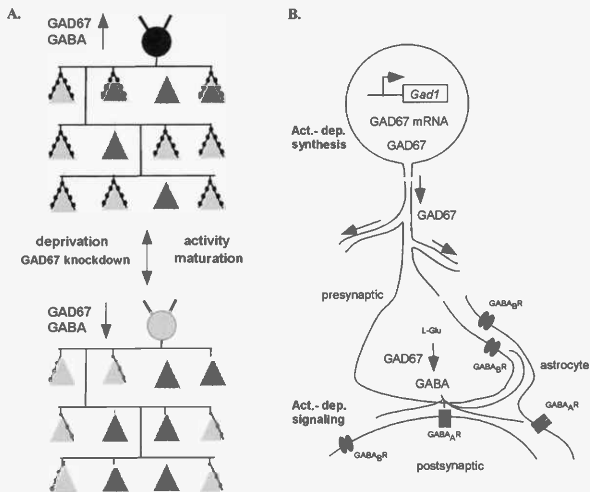

FIGURE 1 (*A*) Perisomatic synapses and innervation field of the basket interneuron influenced by GAD67. (*Top*) A basket interneuron (*large circle*) innervates many pyramidal neurons (*triangles*) with charateristic perisomatic synapses (*black dots*). (*Bottom*) A reduction of GAD67-mediated GABA synthesis by genetic knockdown or activity deprivation leads to decreased axon arbor branching and reduced perisomatic synapse density. (*B*) Activity-dependent GABA synthesis and signaling regulate GABAergic synapse development. At GABAergic synapses, $GABA_AR$ and $GABA_BR$ localize to pre- and postsynaptic and glia components. GABA release regulates synaptic morphogenesis in addition to mediating synaptic transmission (*black arrow*). At basket cell soma, GAD67 production is regulated by input activity, which results in cell-wide adjustment of GABA synthesis and level.

ture of OD plasticity. Using visual cortical slice preparations in which inhibition was left intact, we found that converging layer-4 inputs cooperated to drive spiking in layer-2/3 pyramidal neurons in the third postnatal week, but were antagonistic in the fourth week. Antagonism was mediated via enhanced GABAA receptor signaling which correlated with the increase (peak) of OD plasticity. In contrast, this age-dependent, spike-timing-dependent plasticity (STDP) mechanism was equally potent before and after the onset of OD plasticity. By simulating the antagonistic effect of synaptic inhibition to a model integrate-and-fire neuron, we demonstrated that enhanced synaptic inhibition increases the effectiveness of STDP-driven competition to promote selective weakening of asynchronous inputs. Therefore, maturation of GABAergic inhibition appears to regulate the integration window and coincidence detection of layer-2/3 pyramids in a manner that promotes competition among eye-specific inputs and thus may underlie heightened OD plasticity at the peak of the critical periods (S. Kuhlman, in prep.).

We recently discovered a novel function of PSA-NCAM (polysialic-acid-linked neural cell adhesion molecule) in the maturation of GABAergic innervation and critical period plasticity in the visual cortex. We found that PSA-NCAM expression is regulated developmentally and by visual experience in the postnatal visual cortex, but is inversely correlated to the maturation of GABAergic innervation. Earlier removal of PSA accelerated the maturation of GABAergic inhibition and onset of the critical period. Our results suggest that PSA-NCAM normally "holds

off" the maturation of GABAergic inhibition in the early postnatal visual cortex. Activity-dependent removal of PSA after eye opening enables maturation and promotes plasticity (G. diCristo, in prep.).

GENE EXPRESSION PROFILE AND PROGRAMS IN DIFFERENT CLASSES OF GABAergic INTERNEURONS

Decades of physiological and anatomical studies have revealed stunning heterogeneity and complexity of GABAergic interneurons in their intrinsic and synaptic physiology, morphology, and connectivity. Such complexity is likely conferred by differential gene expression among different cell types, at different developmental stages, and in response to stimulus. A thorough characterization of gene expression profiles among GABAergic interneurons may provide the most comprehensive and quantitative description of their molecular makeup and yield fundamental insight into their classification, biophysical repertoire, and the genetic program directing their development. A bottleneck for such an effort is the ability to purify defined classes of interneurons in sufficient quantity for microarray studies. In collaboration with us, Sacha Nelson and colleagues at Brandeis University have succeeded in developing a method to manually purify GABA neurons for microarray analysis. This method is reliable and sensitive and demonstrated a profound molecular heterogeneity among 12 classes of neurons in the mouse forebrain. We are applying this method to different developmental times in the neocortex and cerebellum. The cerebellum is appealing since various transgenic mice label every major class of GABAergic neuron as well as Bergmann glia cells from early developmental stages. Combined with bioinformatics analysis, these studies will reveal novel biophysical properties, signaling pathways, and transcription programs among interneurons. They should also yield better tools (such as genes that define novel cell types) for more refined genetic manipulations. Such a nonhypothesis-driven approach is essential to elucidate the genetic design of the GABAergic system, its function, and its capacity for plasticity.

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BIOPHYSICAL BASIS FOR NEURAL COMPUTATION

A. Koulakov D. Tsigankov

This year, we continued focusing on the theoretical model for development of connectivity in the visual system. Our model combines the effects of binding and activation of chemical labels, such as Eph receptors and their ligands, ephrins, and Hebbian plasticity. We also included this year the effects of growth factors, such as BDNF, in our model. We studied both the final connectivity configurations and the dynamics of axons and dendrites when this connectivity is formed. Our model allows us to study the role of various factors on neural development and make experimentally testable predictions. We thus showed that in some cases, Hebbian mechanisms can contribute to the formation of neural connectivity with single-neuron precision. General methods advanced by us this year allow us to represent formation of a more abstract neural network as a result of interplay between genetic factors represented by molecular labels and environmental information given by neuronal electric activity. Our theoretical methods will allow us to address a broad range of questions pertaining to the dynamics and configurations of biologically realistic neural networks in the future.

A Unifying Model For Activity-dependent and Activity-independent Mechanisms of Topographic Map Development

D. Tsigankov, A. Koulakov

In developing brains, axons are able to find and recognize their targets based on specific chemical cues that are emitted into intercellular space or localized on the cellular membranes. Further refinement of neural connectivity is dependent on correlated neural activity. To understand the relationship between the effects of chemical labels and activity-dependent refinement, we investigated a quantitative model that is capable of including both of these factors. Our model generalizes Sperry's chemoaffinity principle. We postulate that the system of axons follows a gradient and optimizes the binding affinity of axons to their targets. The affinity includes contributions from binding and activation of chemical labels as well as activity-dependent contributions. Inputs in our model include the distribution of chemical cues expressed by axons and dendrites, the affinity matrix between these cues, and the correlation function of electric activity between connecting cells. This model allows us to obtain the complete connectivity matrix between cells. To test our model, we investigated the formation of topographic connectivity between retina and superior colliculus or optic tectum that depends on binding and activation of the Eph family of receptor tyrosine kinases by their ligands, ephrins. We obtained the following results: (1) Combined effects of chemoaffinity and correlated activity can help to organize connectivity with single-neuron precision. (2) In Isl2/EphA3 knockin mice described recently, the requirements imposed by molecular cues may contradict the activity-dependent Hebbian factors. In these animals, the winning factor depends on the relative strength of these contributions. (3) The axonal branch dynamics observed in the optic tectum of *Xenopus* tadpoles is reproduced in detail by our model. (4) Finally, geometric connectivity inferred from the overlap of dendritic and axonal arbors does not necessarily reflect functional connectivity. Our model therefore suggests a general method to combine disparate contributions affecting neural circuitry (Fig. 1).

Speed Accuracy Trade-off in Olfaction

A. Koulakov [in collaboration with D. Rinberg and A. Gelperin, Monell Chemical Senses Center, Philadelphia]

We report the first direct observation of speed accuracy tradeoff (SAT) in olfaction. We developed a behavioral paradigm in which both the time of odor exposure and the difficulty of the odor discrimination task were controlled by the experimenter. The accuracy of odor discrimination performance increases with the duration of odor exposure, and the rate of this increase is slower for harder tasks. We also present a unifying picture of two previous, seemingly disparate, experi-

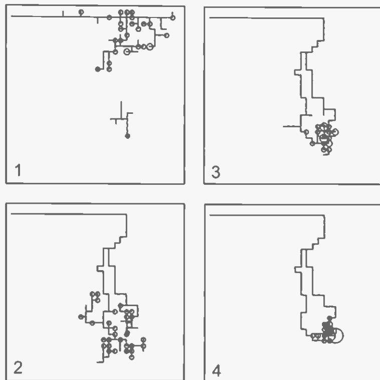

FIGURE 1 Coordinated dynamics of axon, dendrite, and synapses of this axon (*black circles*) in our model. The arbors are shown for four numbered time points. This dendrite and axon end up having 80% of their synaptic connections with each other, which implies a single-neuron innervation. The single-neuron innervation is possible because the arbors of axon and dendrite are highly correlated in geometric space. The soma location determines the level of ephrin expression. This simulation included 900 axons and 900 dendrites (only one pair is shown).

ments studying the timing of rodent odor discrimination. The presence of SAT in olfaction provides evidence for temporal integration in olfaction and constrains the applicability of different models of olfactory information processing.

Combinatorial On/Off Model for Olfactory Coding

A. Koulakov [in collaboration with D. Rinberg and A. Gelperin, Monell Chemical Senses Center, Philadelphia]

We study 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 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 the 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, and (3) extensive lesions of the olfactory bulb do not lead to significant changes in detection/discrimination thresholds. We conclude that a combinatorial code based on a binary glomerular response is sufficient to account for the discrimination capacity of the mammalian olfactory system.

Saha Formula in Olfaction

A. Koulakov [in collaboration with D. Rinberg and A. Gelperin, Monell Chemical Senses Center, Philadelphia]

Olfactory perception is based on interactions between odor molecules and olfactory receptor proteins. The molecular specificity of binding and activation of the receptors by odorants forms the basis for discrimination of smells of different quality and quantity. In this study, we considered the reaction of binding between odor molecules and receptor protein and derived the general relationship for the degree of receptor binding. We show that this relationship has a form of the Saha equation. We argue that the olfactory Weber law may be a consequence of the Saha equation for general assumptions about specificities of odorant-olfactory receptor-binding affinities.

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THE NEURAL ORGANIZATION OF OLFACTORY BEHAVIOR

Z.F. Mainen C.E. Feierstein S. Ranade
G. Felpen D.L. Sosulski
A. Kepecs N. Uchida
M.C. Quirk H.A. Zariwala

Our laboratory is studying the neural mechanisms of goal-directed behavior. We work with a simple but flexible psychophysical paradigm in which rats use odors to guide spatial choices to obtain reward. We are particularly interested in how olfactory information is encoded and transformed into adaptive decisions. We discovered several years ago that rats can respond accurately to sensory information extremely quickly—in a single sniff—and we are continuing to study the temporal limits and mechanisms of rapid olfactory discrimination, as well as more complex computational problems such as concentration- and background-invariant odor recognition and odor-guided navigation. To get at the underlying neural mechanisms, we are using chronic multielectrode recording to monitor single-neuron activity and neural circuit interactions. We recently found that the orbitofrontal cortex (OFC) integrates spatial and reward information to construct maps of behavioral goals. Ongoing recordings focus on temporal coding in the olfactory cortex and the dynamics of representations during learning. From a broader neuroeconomic perspective, we are also studying how the brain deals with the costs of decisions (effort, delay, uncertainty). Finally, we are investigating how psychoactive drugs alter behavior, beginning with ketamine, a drug thought to mimic aspects of psychosis. We hypothesize that drug states can be understood as specific perturbations of neural circuit dynamics. An understanding of neuropharmacology at the circuit level may lead to more effective treatments for disorders such as Parkinson's disease and schizophrenia.

Temporal Integration in Odor Discrimination

H.A. Zariwala, N. Uchida, A. Kepecs

Time can have an important role in the process of deciding between competing options. It is observed that human subjects can sacrifice speed for accuracy in many types of decisions, and a large class of formal decision models is based on the integration of information over time. However, the time scale of integration may vary depending on the nature of the task and the neural systems underlying it.

In a reaction time two-alternative forced choice odor mixture discrimination task (Uchida and Mainen, *Nat. Neurosci. 6:* 1224 [2003]), we found that rats performed the most difficult discriminations with a very small increase in reaction time (~10%) compared to the easiest discriminations. As a follow-up to those observations, our goal has been to motivate rats to use longer odor-sampling times to increase accuracy.

In a first set of experiments, we manipulated task parameters to discourage speed and/or encourage accuracy. We introduced (1) a random delay before odor delivery to prevent motor stereotypy from constraining timing, (2) a long time out or air puff for incorrect choices to increase the cost of errors, (3) a fixed delay (2 sec) from odor onset to reward to deemphasize speed, and (4) a stimulus schedule in which difficult problems were presented in blocks, rather than interleaved with easy problems. We saw no change in either speed or accuracy for the first two manipulations. For the second two, accuracy improved significantly on the hardest problem, but reaction time was not slowed.

In a second set of experiments, we designed a delayed response task by training rats to withhold responding until an auditory "go" signal. This allowed us to enforce a minimum odor-sampling duration more strictly. Using this paradigm, odor-sampling durations increased from approximately 250 msec in the reaction time task to 1000 msec. However, surprisingly, the accuracy on the delay task was not greater than in the reaction time version of the task across all difficulties.

These results show that rapid olfactory reaction times are not simply a question of motivation, but rather, they reveal fundamental underlying neural constraints. Important factors that may favor rapid processing of olfactory information is decreasing information rates due to sensory adaptation and chunking

by the sniff cycle. Coding of elementary olfactory information in discrete snapshots may facilitate higher-order olfactory computations such as associating olfactory cues with spatial landmarks or navigating toward odor sources.

Control of Sniffing: Active Sensation and Olfactory Behavior

A. Kepecs, N. Uchida

Sensory organs are not passive receivers but active instruments controlled by the brain. A familiar example of active sensation is saccadic eye movement—control known to be critical to vision. Olfaction also depends on an active motor process, sniffing, but relatively little is known about how it is regulated during behavior or what role this regulation has in olfactory processing.

A nasal thermocouple (small temperature-sensing probe) allows us to monitor respiration in freely behaving animals. Using this method, we are examining the regulation of respiration in rats performing a two-alternative olfactory discrimination task. Respiration frequency varied over a wide range (<2 to >12 Hz) during the course of a behavioral session, systematically changing in relation to significant behavioral events such as entry into the odor sampling or reward ports. However, when examined in relation to the precise timings of behavioral events, rather than a continuous distribution, discrete frequency modes were observed. In particular, rats switched to a θ frequency (7–10 Hz) mode shortly before odor port entry, remained locked to this mode throughout odor presentation, and then switched to a higher mode (9–12 Hz) before entry into the goal port. Switches between modes occurred very rapidly, almost always within a single respiration cycle. Interestingly, the timing of respiration was related not only to the sensory demands of odor sampling, but also to other motor processes. In particular, the timing of head retractions from the odor and choice ports was precisely coordinated with the respiratory cycle. These observations reveal a powerful and rapid top-down control of respiratory central pattern generators (CPGs), allowing both expectations to direct sensory acquisition as well as the coordination of respiratory and motor processes.

Odor Coding in the Olfactory Cortex

N. Uchida

Our psychophysical studies showed that a single sniff of 150 msec can provide precise information about an odor for a rat (Uchida and Mainen, *Nat. Neurosci. 6:* 1224 [2003]). These findings suggested that the sniff cycle may be a fundamental unit of odor information coding and processing (Kepecs et al. 2005) and raised the question of how olfactory information is encoded on such a time scale. Two candidate mechanisms that might allow especially rapid odor coding are precise timing of spikes in reference to spikes of other neurons (synchrony coding) or timing of spikes relative to the sniffing cycle (latency or phase coding).

To test these ideas directly, we are performing chronic multielectrode recordings of multiple neurons in the olfactory cortex in behaving rats (Fig. 1). Rats are trained to perform a two-alternative olfactory discrimination task using two to three odor pairs. Sniffing is simultaneously recorded using a thermocouple (temperature sensor) implanted in the nasal cavity. Rats are trained to perform at high levels of accuracy, but the stimuli include interleaved binary odor mixtures that

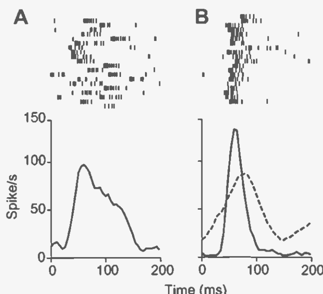

FIGURE 1 Single-unit data from one recording session. These data demonstrate that spikes are indeed precisely locked the sniffing cycle, achieving an instantaneous rate of almost 150 Hz less than 50 msec after sniff onset. (*A*) Response of an olfactory cortical neuron recorded in a behaving rat during sampling of presentation of an odor (ethyl 3-hexenoate). (*Top*) Spike rasters from 20 representative trials. (*Bottom*) Poststimulus time histogram. (*B*) Same data aligned to the first sniff cycle after odor onset (represented by the dotted line). Note tight locking of response to the respiratory cycle.

test the psychophysical limits of performance. Critically, with this paradigm, the efficiency of candidate codes can be assessed by optimal observer decoding in comparison to behavioral performance. Are spike count codes sufficient to explain the psychophysical performance or are spike timing codes necessary?

Single-unit Encoding of Goal-directed Behavior in the Orbitofrontal Cortex

C.E. Feierstein

Every day we face the need to make decisions: We choose between different alternatives to achieve a particular goal. This is the essence of "goal-directed" behavior. Making a decision is a complex process that depends on integrating information about the environment, the alternative actions and their consequences, and choosing what is more likely to result in the accomplishment of our goal.

We are interested in understanding the neural basis of decision making and goal-directed behavior. In particular, we study olfactory-guided decisions. Our studies have focused on the OFC, an area of the prefrontal cortex that forms part of a network of brain regions important in decision making. The most prominent representation described in this area is that of the reward value associated with stimuli.

We are using chronic multielectrode recording techniques to monitor the activity of ensembles of neurons in rats performing a two-alternative odor discrimination task; rats must learn to associate each stimulus with a movement to one of two spatial goals to obtain reward. In this paradigm, we found that a large fraction (56%) of OFC neurons was selective for choice (movement) direction or goal location (left/right). Neurons responded also during and in anticipation of reward delivery or omission. Interestingly, a large subset of the direction selective neurons was jointly selective for the trial outcome (e.g., a cell would fire differently in left choice trials that resulted in an error versus left trials that resulted in a correct response).

These findings demonstrate that circuits in the rodent OFC integrate value information with an objective representation of the spatial environment, thus forming a map of behavioral goals. How do these representations arise in the OFC? We are now recording the activity of neurons in the OFC during learning of new associations, trying to understand the role of this area in olfactory-guided behavior.

Behavioral Impact and Neural Representation of Uncertainty in Perceptual Decision Making

A. Kepecs, N. Uchida

Making good decisions requires the anticipation of the consequences associated with each alternative. Because outcomes are rarely certain, a core component of decision making involves the prediction and evaluation of uncertainty about different options. To study uncertainty in decision making, we are using the standard odor mixture discrimination paradigm developed in our lab.

Rats are trained on binary mixtues of two odors, "A" or "B," in different ratios (100/0, 80/20, 68/32, 56/44, etc.). Rats are rewarded at the left port for mixtures $A/B < 1$ and at the right port for $A/B > 1$. For stimuli near the boundary, $A/B = 1$, there is ambiguity in the relationship of a given odor mixture to the proper choice. Much like asking whether a particular blend of blue and green colors is more blue than green depends on an arbitrary convention of color categories, this training protocol enforces an arbitrary boundary between two odor mixtures. Therefore, there are at least two major sources of decision uncertainty for the rat: variability associated with the encoding of odors (sensory uncertainty) and variability in the memory of the true decision boundary (memory uncertainty).

To explore the neural representation of decision uncertainty, we have recorded from the OFC. Because rewards are fixed in our task (for correct choices), the current value associated with each choice depends directly on the uncertainty of decisions. An accurate estimate of uncertainty should vary with mixture ratio (because rats make more mistakes and hence are more uncertain of odors near the boundary) as well as with accuracy (because, on average, their performance will reflect their uncertainty). Our preliminary findings show that a large fraction of OFC neurons systematically encode mixture ratios, as well as predict choice accuracy during the period of reward anticipation. These observations suggest that orbitofrontal activity provides an estimate of uncertainty.

We therefore wondered if uncertainty actually contributes to decisions made by the rats on a trial-to-trial basis. Because learning that the decision boundary

may require continual updating, we tested whether previous trials affected performances. According to statistical learning theory, the rate of learning should be proportional to uncertainty: For difficult problems (e.g., 56/44 mixture), the outcome is very informative about location of the decision boundary, whereas the outcome of pure-odor trials reveals little about the boundary. Consistent with this, we found that rats biased their decisions to the more recently rewarded direction as if their decision boundary was shifted and the shift was proportional to the uncertainty of the problem. These results suggest that rats possess a representation of uncertainty associated with their decisions and ongoing adjustments in behavior depend on the magnitude of this variable.

How a Delay Affects the Perceived Value of a Reward, Behaviorally and Neuronally

G. Felsen, H.A. Zariwala

Animals make choices based on the perceived values of their associated outcomes. Understanding how value is computed and assigned is thus critical for explaining behavior. One factor that strongly influences value is the duration of the delay that precedes the outcome: The subjective value of a reward decreases as the delay preceding its presentation increases. In other words, given a choice between two rewards of equal magnitude, animals (including humans) will typically prefer the reward associated with the shorter delay and often even select a reward with a smaller magnitude if the alternative is sufficiently delayed. For example, a human subject may opt to receive $10 after 2 minutes rather than $20 after 2 days.

To study this process, known as delay discounting, we are developing a paradigm in which rats freely choose between rewards of differing delays and magnitudes. In our first set of experiments, we obtained a result that would not have been predicted by existing theories: When the delay period is spent where the reward is to be delivered, rats choose to endure longer delays over shorter ones. We speculate that such behavior could result from the formation of a positive association with the location itself (since that is where the reward is ultimately delivered), which overrides any decrease in the reward value due to the longer delay. Although this unexpected finding is interesting in itself, in order to observe behavior influenced by delay discounting, we will modify the task such that the location of reward delivery will be dissociated from where the delay itself is spent.

Ultimately, we would like to use this behavioral paradigm to study how delay is represented in the prefrontal cortex. We plan to record from populations of neurons in the OFC, an area implicated in the subjective assignment of value to rewards, of rats as they perform the behavioral task. Our results will allow us to quantify neuronal selectivity for reward delay and to study how that selectivity evolves and develops during the processes of decision making and attainment of reward.

Recordings from Serotonergic Raphe Nuclei in Awake Behaving Rats

S. Ranade

Serotonin is an important neuromodulator implicated in a range of behavioral, cognitive, and psychiatric disorders. Serotonin is secreted by neurons located in a set of nuclei in the midbrain called the raphe nuclei that send diffuse projections through the entire neuraxis. Although much knowledge of serotonergic function has been gained through pharmacology there are only a few recordings studies from raphe nuclei in awake animals and none in an animal engaged in a task. Therefore, to gain insight into serotonin function, we would like to record from raphe neurons in rats performing a behavioral task. On the basis of reinforcement learning models and a variety of anatomical and pharmacological data, we hypothesize that raphe neuronal firing will encode negative outcomes (e.g., reward omission).

During the past year, we solved most of the technical hurdles involved in recording from raphe nuclei. We have developed a procedure for tetrode drive implantation to reliably target these nuclei. This involves placing a guide cannula through the midline sagittal sinus up to a depth of 3 mm from the skull surface and lowering the drive through the cannula into the brain. This technique allows us to accurately localize fine-wire tetrodes to the dorsal and median raphe nuclei.

We first recorded neurons from these nuclei in awake, freely behaving rats and determined their firing properties. Neurons were classified on the basis of firing rate, spike width, and rhythmic modulation. A subset of high-firing-rate neurons showed rhythmic modulation at θ, β, and/or γ frequencies. A very small pro-

portion of low-firing-rate neurons (<10% of total population) had wide spikes (>400-msec peak to valley), similar to serotonergic neurons reported in literature.

To study responses of raphe neurons during goal-directed behavior, we trained rats on a two-alternative choice odor discrimination task. To test responses of raphe neurons to negative outcomes, we introduced probabilistic reinforcement. In this modified task, we omitted reward on a small proportion (10–20%) of randomly interleaved trials. Omission of expected reward is a negative outcome, and we hypothesize that raphe neurons will show firing-rate modulation in response. Our future goals involve testing our hypotheses of serotonin neuron firing in reinforcement and sniffing.

Inter-areal Coordination among Brain Regions during Olfactory Decisions

A. Kepecs

Much is understood about how individual brain regions represent specialized properties of the environment and perform dedicated processing, but it remains unclear how the brain coordinates its many regions to arrive at coherent decisions. The dynamic coordination of activity is essential for any complex network like the brain, where multiple nodes or regions are needed to accomplish a particular function. For instance, if you were asked to switch from writing down what you are hearing to what you are smelling, your brain would have to dynamically modulate the flow of information between its auditory, olfactory, and motor areas, turning off an auditory-motor pathway and turning on an olfactory-motor pathway. Yet the long-range wiring of the brain is essentially fixed. How then do neural networks in the brain accomplish dynamic and flexible routing of information? Or to put it another way, How does the massively parallel and asynchronously operating brain coordinate its operation?

We are exploring the idea that transient and dynamic oscillatory synchronization between brain regions is used to differentially route and thus coordinate information flow. In particular, we recorded local field potentials (representing the summed coherent activity in local neuronal populations) simultaneously from the several areas relevant to the olfactory task: olfactory bulb, olfactory cortex, and the dorsal and ventral hippocampus while rats performed olfactory discriminations. Strong θ (6–10 Hz, similar to sniffing frequency) oscillations occurred at all recording sites during the task, but only the hippocampal areas were coherent. In contrast, transient β (20–30 Hz) oscillations appeared and became phase-locked between the olfactory bulb and ventral CA1 areas. Around the time of response initiation, the ventral CA1 region showed strong β coherence with the olfactory bulb and, at the same time, θ coherence with the dorsal CA1 region. Thus inter-areal coherence across different brain regions can be frequency specific, and the same brain region can be coherent at different frequency bands with different regions. Currently, we are examining how coherent inter-areal activity is modulated by behavioral contingencies during the task.

Network Dynamics of Cortical Interneuron Subtypes

M.C. Quirk

A distinguishing feature of the neocortex is its cellular diversity. Consequently, a prerequisite for understanding the computations performed by cortical circuits is the ability to identify and study distinct cell classes. This issue is particularly acute in studies of cognitive function in behaving animals where access is generally limited to extracellular recordings. To address this problem, we have developed methods for distinguishing physiologically distinct neurons in chronic multielectrode recordings from the neocortex of behaving rats.

Using unsupervised clustering algorithms applied to a set of extracellular waveform and spike interval metrics, we have been able to identify both excitatory pyramidal cells and two classes of inhibitory interneurons, termed NS1 and NS2. By comparing our data with previous in vitro studies, NS1 neurons appear to correspond to a relatively homogeneous class of somatically targeting interneurons called basket cells, whereas NS2 cells correspond to a more diverse set of interneurons that generally provide dendritic inhibition.

Although inhibitory interneurons have been extensively studied in vitro, little is known of their properties in vivo. We find that NS1 and NS2 neurons display distinct network dynamics. NS1 neurons are well situated to provide both feedback and lateral inhibition to neighboring pyramidal cells. The firing activity of spatially separated NS1 neurons is also highly synchronized, suggesting that the NS1 neurons have an impor-

tant role in coordinating the output of spatially distributed but functionally related cell populations. In contrast, the network properties of NS2 neurons are more varied, suggesting that these neurons participate in a diverse host of yet unknown processes. Preliminary behavioral analysis further suggests distinct computational functions of NS1 and NS2 neurons.

Given that somatic and dendritically targeting interneurons exhibit differential and selective vulnerability across a variety of neurological and psychiatric states, the ability to identify distinct classes of interneurons in vivo will provide an important avenue for defining neural circuit endophenotypes of psychiatric diseases.

Neural Circuit Mechanisms Underlying the Ketamine-Induced Dissociative State

D.L. Sosulski, M.C. Quirk

Dissociatives are a class of drugs that includes ketamine, phencyclidine (PCP), and dizocilpine (MK-801). At high doses, these drugs produce anesthesia and amnesia. At lower doses, they can produce a form of altered consciousness in which cognitive activity is disconnected from sensory information processing. At these subanesthetic doses, dissociatives have been proposed to model acute psychosis. From a clinical perspective, ketamine can mimic not only the "positive" clinical symptoms of schizophrenia (e.g., delusions), but also the "negative" symptoms (e.g., social withdrawal) and impairment of cognitive functions (e.g., working memory).

The dissociative state can be understood as the mental and behavioral correlate of a specific form of altered neuronal dynamics produced by the action of this class of drugs. A characterization at the neuronal level of the phenomenology of this state and a better understanding the mechanisms that produce it should provide new insight into the mechanism of action of psychoactive drugs and could help to define neural endophenotypes of schizophrenia.

To address these issues, we are examining the effect of ketamine on neural activity in the prefrontal cortex of behaving rats. Chronic multielectrode recording techniques are used to isolate ensembles of single neurons in the behaving animal. Using the techniques described above ("Network dynamics..."), we identified putative fast-spiking interneurons and pyramidal neurons. Rats are given daily injections alternating between ketamine (30 or 7 mg/kg) and control solution (1% saline).

We find that ketamine, at both 7 and 30 mg/kg doses, produces a robust suppression of interneuron firing. Pyramidal neuron firing rates are also altered but show mixed effects, with approximately half increasing and half decreasing. We are currently using measures of oscillatory synchrony as a means to further characterize the circuit dynamics produced by dissociatives.

Our findings begin to define the nature of a psychosis-like state at the level of single neuron activity. We suggest that ketamine, an NMDA (*N*-methyl-D-aspartate) receptor antagonist, acts either directly or indirectly by disrupting the activity of one or more classes of interneurons, probably localized to the prefrontal cortex. This in turn appears to cause a broader disorganization of neuronal activity in the pyramidal cell population, the characteristics of which remain to be defined. These observations lend support to the theory that the symptoms of schizophrenia may reflect alterations in brain function resulting from dysregulation of interneuron activity.

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TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow W. Benjamin, J. Boehm, N. Dawkins-Pisani, I. Ehrlich, H. Hsieh, H. Hu, H. Kessels, C. Kopec, B. Li, T. Takahashi, W. Wei

My laboratory is directed toward understanding synaptic function, synaptic plasticity, and synaptic dysfunction. Through such an understanding we hope to elucidate how learning and memory are achieved and how diseases corrupt them. This year, we continued to examine the regulation of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and *N*-methyl-D-asparate (NMDA) receptors at synapses that may underlie plasticity and the malfunction that could lead to diseases. Some of our studies are summarized below.

AMPA-R Removal Underlies Aβ-induced Synaptic Depression and Dendritic Spine Loss

H. Hsieh, R. Malinow [in collaboration with S. Sisodia, University of Chicago]

Beta amyloid (Aβ), a peptide generated by neurons, is widely believed to underlie the pathophysiology of Alzheimer's disease when overproduced. Recent studies indicate that this peptide can drive endocytosis of AMPA- and NMDA-type glutamate receptors. We now show that Aβ uses signaling pathways of long-term depression (LTD) to drive endocytosis of synaptic AMPA receptors. Synaptic removal of AMPA receptors is key, because it is necessary and sufficient to drive loss of dendritic spines and synaptic NMDA responses. Our results indicate that increased levels of Aβ tap into endogenous physiological processes to depress synaptic structure and function.

PSD-95 Is Required for Activity-driven Synaptic Development

I. Ehrlich, R. Malinow

The activity-dependent regulation of AMPA-type glutamate receptors and the stabilization of synapses are critical to synaptic development and plasticity. One candidate molecule implicated in maturation, synaptic strengthening, and plasticity is PSD-95. Here, we find that acute knockdown of PSD-95 in brain slice cultures by RNA interference arrested activity-driven development of synaptic structure and function, manifested by reduced synaptic strength and altered spine morphology. Surprisingly, PSD-95 was not necessary for the induction and early phase of long-term potentiation (LTP) expression. However, following PSD-95 knockdown, chemically induced LTP produced smaller changes in the size of stable spines, and we observed a larger fraction of transient spines that turned over more readily. Taken together, our data support a model in which PSD-95 is required for activity-dependent synapse stabilization following initial phases of synaptic potentiation.

Signaling by *Neuregulin/ErbB4*, Genes Implicated in Schizophrenia, Is Critical to Proper Maturation of Excitatory Synapses

B. Li, R. Malinow [in collaboration with L. Mei, College of Georgia]

Neuregulin 1 (*NRG1*) and its receptor *ErbB4* activate signaling cascades that are essential for the development and function of many organ systems including the neural system. However, the function and mechanism of *NRG1* and its receptors in the central glutamatergic synapse are poorly understood. Here, we show that *NRG1* signals via *erbB4* to control spine growth, synaptic maturation, and synaptic plasticity at the Schaffer collateral-CA1 synapse. Our data suggest that the defect in glutamatergic synapse development caused by a perturbation in the *NRG1/erbB4* signaling pathway may contribute to the etiology of schizophrenia.

Postsynaptic Receptor Trafficking Underlying a Form of Associative Learning

S. Rumpel, A. Zador, R. Malinow [in collaboration with J. LeDoux, New York University]

To elucidate molecular, cellular, and circuit changes that occur in the brain during learning, we investigated the role of a glutamate receptor subtype in fear conditioning. In this form of learning, rodents associate two stimuli, such as a tone and a shock. In this study, we found that fear conditioning drives AMPA-type glutamate receptors into the postsynapse of a large fraction of neurons in the lateral amygdala, a brain structure essential to this learning process. Furthermore, memory was reduced if AMPA-receptor synaptic incorporation was blocked in as few as 10–20% of lateral amygdala neurons. Thus, the encoding of memories in the lateral amygdala is mediated by AMPA-receptor trafficking, is widely distributed, and displays little redundancy. We have continued these studies by examining dendritic spines in vivo before and after fear conditioning.

Spine Enlargement Precedes AMPA-receptor Exocytosis during LTP

C. Kopec, B. Li, W. Wei, J. Boehm, R. Malinow

The changes in synaptic morphology and receptor content that underlie neural plasticity are poorly understood. In this study, we used a pH-sensitive green fluorescent protein (GFP) to tag recombinant glutamate receptors and showed that chemically induced LTP drives robust exocytosis of AMPA receptors onto dendritic spines. In contrast, the same stimulus produced a small reduction of NMDA receptors from spines. LTP produced similar modification of small and large spines. Interestingly, during LTP induction, spines increased in volume before surface incorporation of AMPA receptors, indicating that distinct mechanisms underlie changes in morphology and receptor content. We are continuing these studies by establishing optical methods to detect synapses that have undergone plasticity.

Differential Subcellular Overexpression of AMPA-receptor Subunits in CA1 Neurons

H. Kessels, M. Klein, R. Malinow

The role of AMPA-type glutamate receptors in synaptic plasticity can be studied by the introduction of recombinant AMPA subunits in glutamatergic neurons. Here we show that, whereas transient expression of AMPA subunits leads to considerable overproduction in neuronal somata of organotypic hippocampal slices, at dendrites they remain close to endogenous levels. These results provide evidence for a tightly controlled transport mechanism of AMPA-R complexes traveling from soma to dendritic compartments. We have now evidence that the AMPA-R associated protein, stargazin, is a rate-limiting protein in the transport of AMPA-Rs from cell bodies to dendrites. This transport system is selective for certain AMPA-R subunits.

Direct Phosphorylation by PKC at a Novel Site on AMPA-R Subunit Glur1 Controls Synaptic Incorporation during LTP

J. Boehm, R. Malinow [in collaboration with R. Huganir, Johns Hopkins University]

Incorporation of GluR1-containing AMPA-Rs into synapses has an important role in several forms of neural plasticity. A number of signaling pathways have been identified, but the direct modifications of GluR1 that control its synaptic incorporation have not yet been found. Previous studies indicate that activation of protein kinase C (PKC) is required to generate LTP, a leading model of synaptic plasticity. However, the targets of PKC responsible for LTP have not been determined. We find that GluR1 Ser-818, which is highly conserved evolutionarily, is phosphorylated by PKC in vitro and by LTP in hippocampal slices. Acute phosphorylation by PKC at GluR1 Ser-818 as well as molecular mimicking phosphorylation at this site drives GluR1 into synapses. Preventing GluR1 Ser-818 phosphorylation blocks LTP and PKC-driven synaptic incorporation of GluR1. Thus, GluR1 Ser-818 is rapidly phosphorylated by PKC during LTP induction and is required for its incorporation into synapses.

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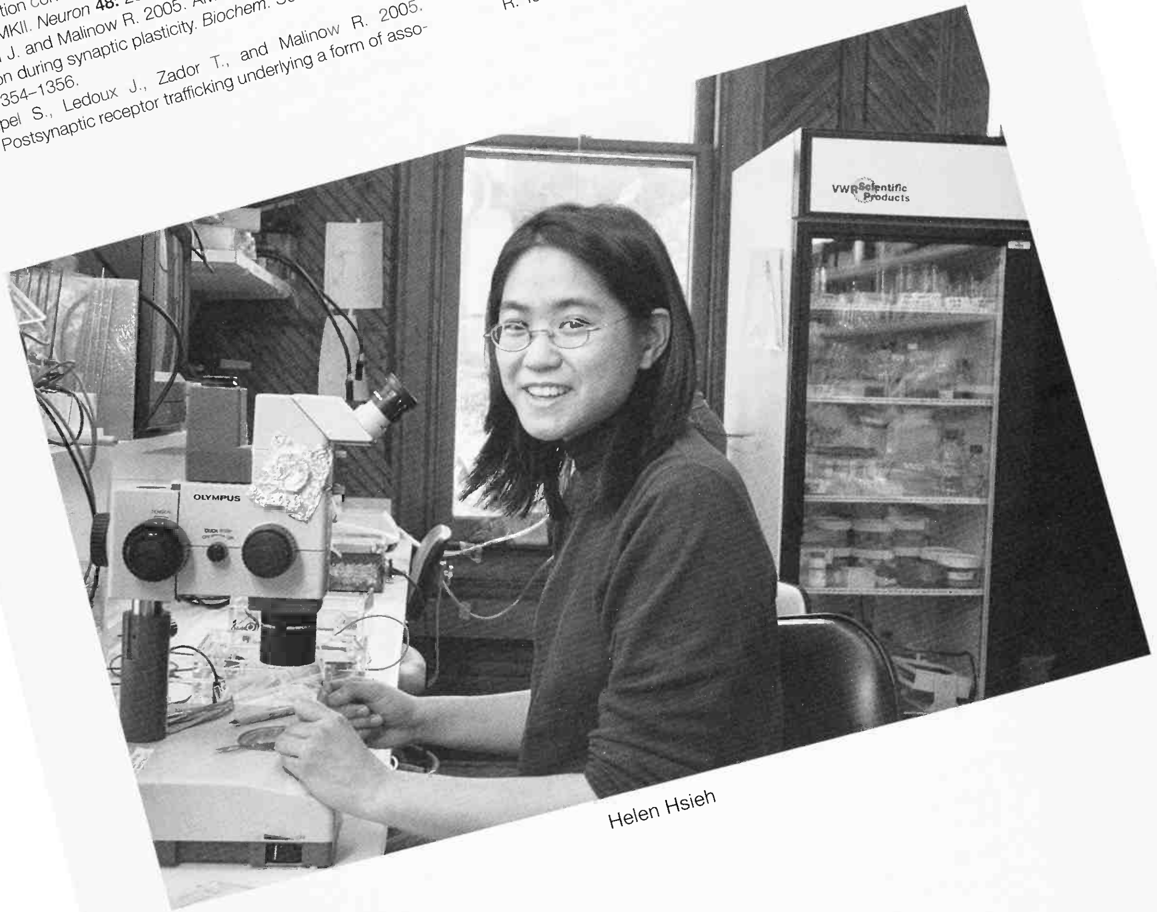

Helen Hsieh

INTEGRATIVE SYSTEMS NEUROBIOLOGY

P. Mitra P. Andrews H. Maniar
H. Bokil A. Puniyani

Our basic research philosophy is to bring theoretical, computational, and experimental tools to bear on biological questions, in general, and neurobiological questions, in particular, to make sense of biological complexity and provide understanding and potential therapies for disease. There are three basic branches to the research: Neuroinformatics, Theoretical Engineering, and Quantitative Behavior and Electrophysiology. The first involves the application of statistical and signal processing tools to large volumes of neurobiological data. This continuing research effort, reflecting several years of work, has given rise to the Chronux analysis platform and is associated with the Neuroinformatics summer course 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, both in terms of the environment and the 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. The third component of the research is experimental and includes behavioral and electrophysiological studies in multiple species including the zebra finch, the macaque, and more recently, *Drosophila*.

Our laboratory personnel includes Hiren Maniar, a scientific informatics analyst working as part of a consortium funded by the Human Frontiers Science Foundation on the role of neural synchrony in cross-modal integration; Hemant Bokil, a postdoctoral fellow continuing his work on signal processing applications to neurobiological time series with special emphasis on neural prostheses; and Peter Andrews, a scientific informatics manager responsible for the hardware and software infrastructure for the group; he is also in charge of the development and maintenance of the Chronux software platform for neural signal processing. Hemant and Hiren are working with Peter on the development of Chronux. Amit Puniyani is one of two newly hired postdocs working on the DART project.

Apart from this group of core personnel, our research effort includes close collaborative ties with multiple research groups. A collaborative effort with Niko Schiff and Keith Purpura at the Weill Medical School of Cornell University, where I am an adjunct associate professor, involves electrophysiology in the awake behaving macaque. A second research effort on how zebra finches learn their song vocalizations is being carried out in collaboration with a consortium of zebra finch researchers based in four different universities, including a long-standing collaboration with Ofer Tchernichovski at City College of New York. This collaboration has been fruitful, with a publication earlier in 2005 on the effects of sleep on song development in the zebra finch. A third collaboration is with Rodolfo Llinas's group at New York University Medical College. 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) and Pascal Fries (F.C. Donders Research Center at Nijemegen). A fifth project started in 2005 is the integrative analysis of memory formation in the fruit fly, as part of the Dart Neurogenomic Alliance at CSHL led by Tim Tully.

Chronux: Open Source Software for Neural Signal Processing

P. Andrews, 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 past 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 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. We expect that tools such as these will be critical to advancing our understanding of systems neuroscience.

Substantial improvements have been made to Chronux. The Chronux Matlab toolbox has now been released under the open-source software license GPL V2, which we hope will promote more external contributions to the project. Of significant interest is the recent incorporation of the entire C Locfit library by Catherine Loader, a statistics expert at Case Western Reserve University, as a set of Matlab mex functions. Locfit adds to Chronux the ability to fit curves and surfaces to data using local regression and likelihood methods. Additional algorithms added to Chronux include nonstationary spectral analysis techniques, and an algorithm for comparing spectra or coherences from two groups of trials with unequal sizes that we have recently developed. These additions are aimed at problems that arise frequently in neural signal processing.

LBEX: A Toolbox for Source Localization in MEG

H. Maniar

Magnetoencephalography (MEG) is the noninvasive imaging of brain activity through measurements of the weak magnetic fields generated by this activity. Utility of this imaging modality is contingent on solving an inverse problem to perform source localization. Localization aims to spatially pinpoint the neural activity within the head using externally measured recordings. During the past decade, localization has been used not only to understand the temporal aspects of brain dynamics, but also to chart out various human cognitive abilities. In recent years, localization is also increasingly being used in clinical settings for presurgical planning of epileptic cases.

Various localization techniques are currently employed by researchers, but not all perform satisfactorily. A significant degree of interdisciplinary knowledge is required in conceiving new algorithms. We have developed a new source localization methodology called local basis expansions (LBEX). The technique can be viewed as a spatial analog of multitaper spectral estimation in time-domain, which is the principal methodology we employ to perform neural signal processing. Our goal is to provide neuroscientists with a sophisticated open-source toolbox for reliable source localization of neural activity.

Researchers increasingly believe that gamma-band synchronization in the human brain fundamentally involves several functions including visual feature binding, perceptual and attention stimuli selection, visuomotor control, working memory, and associative learning. Figure 1 shows the results of applying the LBEX technique to MEG data from the laboratory of Pascal Fries (Radboud University, Nijmegen). The data are taken from an experiment in which the subject performs a visual attention task. The figure shown superimposes the power change in a subject's gamma-band activity with visual attention, as evaluated by LBEX, with the subject's structural MRI.

Integrative Analysis of Memory Formation

P. Mitra, A. Punyani

This is the first project year of the Mitra component of the Dart Alliance at CSHL. The broad goal of the Mitra component is integrative data analysis and modeling of memory formation in the fruit fly, spanning scales including genetic, cellular, and neural systems as well as behavioral levels. This represents the principal theoretical modeling component of the alliance. The work so far has been in two areas. The first of these involves microarray data analysis, and the second involves theoretical studies relating to experimental design for a project being carried out by another member of the Dart Alliance (Josh Dubnau). The goal of the selection study (Dubnau and Altick) is to breed flies with better performance on an odor memory task while allowing combinatorial changes to take place in multiple loci simultaneously. This will help elucidate the network of genes that underlie the function being studied. The study poses numerous theoretical questions, including those of efficient experimental design, which we are pursuing actively.

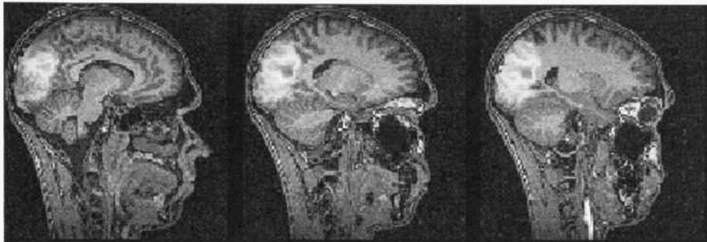

FIGURE 1 Localization of gamma-band activity in a visual attention task using the LBEX technique.

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EXPERIENCE-DEPENDENT PLASTICITY OF SYNAPSES AND SYNAPTIC CIRCUITS

K. Svoboda	B. Burbach	A. Holtmaat	V. Scheuss	L. Wilbrecht
	I. Bureau	A. Karpova	G. Shepherd	R. Yasuda
	V. DePaola	T. O'Connor	A. Sobczyk	C. Zhang
	N. Gray	L. Petreanu	G. Tervo	H. Zhong
	C. Harvey	T. Sato	R. Weimer	K. Zito

The functional properties of the brain 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. We are dissecting the mechanisms 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. Two-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, where 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.

Function and Plasticity of Single Synapses

V. Scheuss, A. Sobczyk, K. Zito

Long-term changes in synaptic efficacy are triggered by NMDA (*N*-methyl-D-aspartate) receptor-mediated increases in [Ca^{2+}] in the postsynaptic neuron. On the basis of 2-photon imaging and 2-photon glutamate uncaging, we have developed quantitative assays to measure NMDA-R (receptor) function at single synapses. Using these techniques, we have found that NMDA-Rs undergo a novel form of activity-dependent plasticity involved with changes in ion selectivity. We are also measuring the time course with which new synapses acquire different types of glutamate receptors.

[Ca^{2+}] Dependent Signaling in Single Dendritic Spines

C. Harvey, R. Yasuda, H. Zhong

Postsynaptic Ca^{2+} activates diverse Ca^{2+}-dependent signal transduction mechanisms. We have designed high-sensitivity fluorescence resonance energy transfer (FRET) sensors for Ras and MAPK activation and cAMP levels. Fluorescence lifetime imaging microscopy (FLIM) allows us to image the Ca^{2+}-dependent signal transduction cascades activated by synaptic transmission in dendrites and spines. We can for the first time study the biochemical dynamics of Ca^{2+}-dependent Ras signaling in neuronal microcompartments. For example, we find that Ras acts as a Ca^{2+}-dependent switch and that activation of single synapses can lead to local activation of Ras. Ras activation is required for spine growth.

Experience-dependent Plasticity in the Adult Cortex In Vivo

V. DePaola, A. Holtmaat, L. Wilbrecht [in collaboration with G. Knott, Lausanne]

Sensory representations in the adult brain are stable, yet we are able to learn. To understand the underpinnings of stability and plasticity, we image structural dynamics in vivo. We find that the large-scale arboriza-

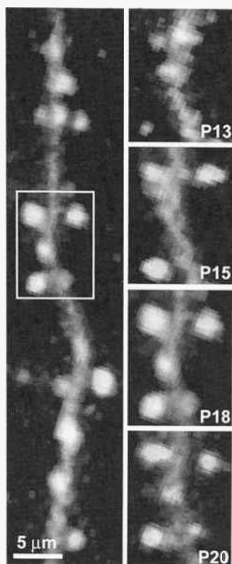

FIGURE 1 Stability of PSD-95 clusters in the intact mouse neocortex. A sparse subpopulation of layer 2/3 neurons expressed a red cytoplasmic protein (mCherry1) and PSD-95 fused to a green fluorescent protein (GFP). (*Left*) Overview of a dendritic branch; (*right*) time-lapse imaging of a region of interest (white box in the left image; ages are indicated in the panels). Note the stable relative sizes of the bright spots indicating stable relative synapse sizes (N. Gray, R. Weimer, K. Svoboda).

tion of axons and dendrites is stable, but that neurons display a rich repertoire of micrometer-level structural plasticity of dendritic spines, axonal terminals, and axonal branch tips. Experience-dependent changes in spines and boutons are cell-type specific. By combining in vivo imaging with retrospective serial section electron microscopy, we have found that new spines grow to make synapses and that they preferentially grow toward existing boutons in the neuropil.

Mechanisms of Synapse Stability In Vivo

N. Gray, R. Weimer

We have recently turned our attention to synapse stability. Many synapses persist for months, perhaps the entire life of the animal, and also maintain their size. This is remarkable because synapses are tiny structures composed of just a handful of proteins of a given type. Protein lifetimes are on the order of days. To study the underpinnings of synapse stability, we have developed methods to measure the trafficking of synaptic proteins at the level of individual synapses in vivo. We find that the major scaffolding protein PSD-95 unbinds from synapses over tens of minutes and exchanges with proteins in neighboring synapses (Fig. 1). Larger synapses scavenge diffusing PSD-95 more efficiently and also hold on to PSD-95 longer. The interactions between synapses and their proteins are thus tuned to maintain the synaptic status quo against dissipation by diffusion.

Circuit Mechanisms of Cortical Plasticity and Dysfunction

G. Shepherd, I. Bureau, A. Karpova, L. Petreanu [in collaboration with M. Chklovskii, Cold Spring Harbor Laboratory]

The wiring diagram is fundamental to understanding cortical function and plasticity. However, little *quantitative* information about *functional* circuits is available. What are the sources of input to a neuronal subtype in a particular layer and column and what are their relative strengths? Which connections change with novel sensory experience?

We have developed laser-scanning photostimulation (LSPS) into a quantitative and rapid tool for circuit analysis. In brain slices, we mapped the excitatory circuits impinging onto L2/3 and L5B neurons. To determine if morphology can predict functional circuits between excitatory neurons, we directly compared functional LSPS maps with "geometric circuits" computed from quantitative reconstructions of axons and dendrites. Functional connections were accurately predicted by geometry within a particular projection (with interesting exceptions), but the ratio of functional to geometric connectivity differed greatly (>20-fold) between projections. This finding implies new forms of specificities in cortical circuits.

We performed an unbiased search for experience-dependent synaptic pathways impinging onto two types of L5B pyramidal cells: regular spiking and intrinsically bursting. Experience-dependent changes in excitatory L2/3→L5B synapses were cell-type specific. Whereas regular-spiking neurons lost input from their home column in an experience-dependent man-

ner, intrinsically bursting cells gained input from surround columns.

We have used LSPS to explore the circuit and defects in animal models of fragile-X mental retardation. We found specific defects in the function and plasticity of excitatory L4→L2/3 synapses.

Microorganization of the Sensory Cortex

T. Sato

We are using 2-photon microscopy combined with Ca^{2+} imaging to measure the activity in neuronal populations in the intact neocortex. Neurons are labeled with a membrane-permeable synthetic Ca^{2+} indicator. Ca^{2+} transients evoked by action potentials can be detected as sawtooth-shaped fluorescence changes. We have developed algorithms to detect and count individual spikes based on Ca^{2+} imaging. Our techniques allow us to study the firing of populations of neurons with well-defined spatial relationships. We are using these tools to learn about the microorganization of cortical maps in the somatosensory cortex and how this organization is shaped by changes in sensory experience.

Molecular Methods to Reversibly Inactivate Synapses In Vivo

G. Tervo, A. Karpova, N. Gray, B. Burbach

Inducible and reversible silencing of selected neurons in vivo is critical to understanding the structure and dynamics of brain circuits. We have developed molecules for inactivation of synaptic transmission (MISTs) that can be genetically targeted to allow the reversible inactivation of neurotransmitter release. MISTs consist of modified presynaptic proteins that interfere with the synaptic vesicle cycle when cross-linked by small-molecule "dimerizers." MISTs based on the vesicle proteins VAMP2/synaptobrevin and synaptophysin induced rapid (~10 min) and reversible block of synaptic transmission in cultured neurons and brain slices. In transgenic mice expressing MISTs in Purkinje neurons, administration of dimerizer reduced learning and performance of the rotarod behavior. MISTs allow for specific, inducible, and reversible lesions in neuronal circuits and may provide treatment of disorders associated with neuronal hyperactivity. We are now constructing knock-in mice expressing MISTs in specific neuronal populations.

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NEUROGENETICS OF MEMORY IN *DROSOPHILA*

T. Tully J. Barditch J. Luo
F. Bolduc M. Regulski
L. Grady N. Sinha
J. Kui S. Xia
D. Leong

Studies on Pavlovian learning in many animals have revealed remarkably similar behavioral properties of associative learning. Surprisingly, Pavlovian learning in fruit flies also displays these behavioral properties, suggesting a common underlying mechanism. Neural architecture of the *Drosophila* brain bears no resemblance to mammalian brains, of course, which suggests that the behavioral "homology of function" must result from conserved cellular/molecular mechanisms. Consistent with this view, several genes and genetic pathways have been identified to function in both vertebrate and invertebrate-associative learning, including NMDA (*N*-methyl-D-aspartate) receptors, cAMP signaling, CREB-dependent gene transcription, and *staufen*-mediated local regulation of protein translation.

We have recently investigated the relationship of associative learning (plasticity) to olfactory discrimination. We have designed three novels assays, which nevertheless are based on the original choice behavior of our standard T maze. These assays are designed to distinguish behavioral responses to odor identity from behavioral responses to odor intensity. In addition, these responses can be assessed in naïve or trained (Pavlovian olfactory conditioning) flies. Our results reveal a genetic dissection of G-protein-mediated signaling pathways between odor identity and odor intensity in mushroom bodies. More interestingly, our data suggest that associative mechanisms induced by conditioned discrimination between two odors, in fact, occur via changes in the perception of odor intensity, rather than odor identity, as has been widely assumed by learning theorists. Consistent with this view, conditioned discrimination between two different odors disappears as odor concentrations are increased to saturating levels (Fig. 1).

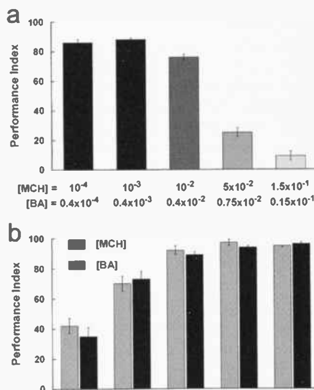

FIGURE 1 Odor discrimination learning fails as odor concentrations increase. (*a*) Conditioned discrimination between two odors (methycyclohexanol [MCH] and benzaldehyde [BA]) declines to zero as odor concentrations increase to saturating levels. (*b*) When given a choice between each of these odors and air, in contrast, odor avoidance responses increase as odor concentrations increase, demonstrating the flies' ability smell each odor.

MUTANTS AND MICROARRAYS

We continue work derived from earlier experiments on regulated gene expression induced during memory formation. To date, we have confirmed 59 of 170 candidate memory genes (CMGs). Our goal is to confirm in vivo that these CMGs are involved in adult memory formation. Using RNA interference or other dominant-negative transgenic constructs, we have obtained in vivo validation for three genes, including *dNR1*.

CIRCUITS AND PHASES

We continue spatiotemporal experiments, which reveal where in the adult brain CREB-dependent memory formation occurs. To date, we have determined that the upstream activation sequence (UAS)-driven expression of a CREB-repressor transgene,

using several different mushroom body GAL4 drivers does NOT block long-term memory (LTM) formation. Conversely, we have identified several enhancer-GAL4 drivers expressing UAS-CREB-r in other brain regions that do block spaced training-specific 1-day memory (LTM). We currently are attempting to understand the circuitry in common among these various GAL4 drivers.

We have established a reliable new method to cell-sort green fluorescent protein (GFP)-expressing neurons from dissociated adult brains. To maximize the protocol, we expressed GFP in mushroom bodies and sorted dissociated cells into GFP$^+$ versus GFP$^-$ subsets. With these conditions, QPCR (quantitative polymerase chain reaction) reveals (1) preferential expression in mushroom bodies of *fasII* and *rutabaga* and (2) no preferental expression in mushroom bodies of *dCREB2* and *AdfI*. These observations are consistent with published immunohistochemical observations.

GENETIC MODELS OF DISEASE

In vertebrate systems, STAUFEN is known to associate with Fragile-X protein (FMRP) in neural granules. On the basis of this observation, we have begun to study the fly homolog of FMRP. To date, we have shown that *FMR1* mutants are defective in olfactory learning and that this learning deficit can be rescued by transgenic expression of a genomic construct. We continue to evaluate an acute role for FMR1 in memory formation.

We are overexpressing human α-synuclein in dopaminergic cells as a fly model of Parkinson's disease. Interestingly, we have shown that tyrosine hydroxylase (TH) immunostaining disappears in an age-related manner, *before* dopaminergic neurons die. This observation supports our ongoing microarray experiments to identify α-synuclein-induced changes in genome regulation that may underlie neurodegeneration.

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NEURAL SUBSTRATE OF SELECTIVE AUDITORY ATTENTION IN THE CORTEX

A. Zador H. Asari, M. DeWeese, T. Hromadka, E. Kramer, S. Lima, G. Otazu, H. Oviedo, S. Rumpel, L.-H. Tai, M. Wehr, Y. Yang

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 also computational approaches to characterize the properties of natural sounds. Second, we are interested in how these representations are modified dynamically—within seconds to hours or longer—in awake behaving rats by attention and other forms of learning.

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; however, 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. (DeWeese et al. 2005).

Sparse Synchronous Inputs Drive Neurons in the Auditory Cortex In Vivo

M. DeWeese, T. Hromadka

Cortical neurons receive most of their synaptic drive from other cortical neurons. This fact imposes a strong constraint on models of cortical activity: The dynam-

ics of one neuron's spiking output must be consistent with the ensemble activity of the population of neurons that synapse onto it. The usual solution to this self-consistency constraint posits that both excitatory and inhibitory neurons fire asynchronously, varying their rates only slowly. According to this model, subthreshold fluctuations in membrane potential should be well described by a random walk. However, on the basis of our experiments using the in vivo whole-cell patch-clamp technique, we have developed an alternate model in which inputs are organized into synchronous volleys. According to this model, subthreshold fluctuations should be small most of the time, punctuated by large excursions corresponding to the arrival of volleys. Our results show that activity is organized into synchronous volleys superimposed on a relatively quiet background. This observation has implications for coding and computation. In particular, the correlations we have found among the synaptic population are precisely what is required for sparsely encoded signals to successfully propagate from one stage of cortical processing to the next.

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 attend selectively 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 multielectrode recording (tetrode) technology to monitor the activity of many neurons simultaneously in awake, behaving rodents performing an auditory discrimination task.

Synaptic Mechanism Underlying Tone-Shock Learning

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 α-amino-3-hydroxy-5-methyl-3-isoazole (AMPA) receptors (AMPA-Rs) 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. To elucidate molecular, cellular, and circuit changes that occur in the brain during learning, we investigated the role of a glutamate receptor subtype in fear conditioning. In this form of learning, animals associate two stimuli, such as a tone and a shock. We have found that fear conditioning drives AMPA-type glutamate receptors into the postsynapse of a large fraction of neurons in the lateral amygdala, a brain structure essential for this learning process. Furthermore, memory was reduced if AMPA-R synaptic incorporation was blocked in as few as 10–20% of lateral amygdala neurons. Thus, the encoding of memories in the lateral amygdala is mediated by AMPA-R trafficking, is widely distributed, and displays little redundancy. We are currently extending these results to the auditory cortex (Rumpel et al. 2005).

Synaptic Mechanisms of Forward Masking in Rat Auditory Cortex

M. Wehr

In the auditory cortex, brief sounds elicit a powerful suppression of responsiveness known as forward masking. Forward masking has usually been attributed to synaptic (GABAergic) inhibition. We are using whole-cell recordings in vivo to assess the role of synaptic inhibition to forward masking in the auditory cortex. We are measuring the excitatory and inhibitory synaptic conductances elicited by brief sounds presented at intervals from tens to hundreds of milliseconds. We find that inhibitory conductances rarely last longer than 50–100 msec, whereas spike responses and synaptic inputs remain suppressed for hundreds of milliseconds, indicating that at these longer intervals, mechanisms other than inhibition dominate. We conclude that postsynaptic inhibition contributes to forward masking for only the first 50–100 msec after a stimulus and that long-lasting suppression in cortical cells is instead due to other mechanisms such as synaptic depression (Wehr and Zador 2005).

Sparse Representations for the Cocktail Party Problem

H. Asari [in collaboration with B. Pearlmutter, Cold Spring Harbor Laboratory]

To extract the behaviorally relevant information imbedded in natural acoustic environments, animals must be able to separate the auditory streams that originate

from distinct acoustic sources (the "cocktail party problem"). The auditory cortex has several orders of magnitude more neurons than does the cochlea, so that many different patterns of cortical activities may faithfully represent any given pattern of cochlear activity. We have been exploring the hypothesis that the cortex exploits this excess "representational bandwidth" by selecting the sparsest representation within an overcomplete set of features. We have constructed a model showing how sparseness can be used to separate sources perceived monaurally. The model makes testable predictions about the dynamic nature of representations in the auditory cortex. Our results support the idea that sparse representations may underlie efficient computations in the auditory cortex.

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Gonzalo Otazu and Lung-Hao Tai

NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong H.-C. Chiang I. Hakker F. Hannan I. Ho K. Iijima A. Mamiya M. Pagani Y. Wang

We are interested in the neural basis of learning and memory and are taking an approach of combining functional analyses with 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 have shown that different fragments of NF1 are involved in regulation of different signal transduction pathways and have distinct roles in mediating formation of short-term memory versus long-term memory (LTM). For Alzheimer's disease, we have shown that overexpression of Aβ peptides in *Drosophila* is able to recapitulate many aspects of clinical manifestations, including age-dependent learning defects, late onset of neurodegeneration, and cumulation of Aβ deposits. We are now attempting to understand the molecular basis for Aβ toxicity. Second, we are making efforts in an attempt to visualize memory traces in the mushroom body, a brain structure critical for insect learning and memory through calcium imaging in the living fly brain. The specific projects are described below.

NF1 Is Involved in Learning and Memory Processes

I.S. Ho, F. Hannan, I. Hakkar, M. Pagani

NF1 is a dominant genetic disorder that affects 1 in 3500 individuals. Besides the predisposition to malignant and benign tumors of the nervous system, 40% of the afflicted children have learning defects. Studies with $Nf1^{+/}$ mice have shown that they exhibit deficit in the Morris water maze paradigm that can be rescued by lowering Ras dosage either genetically or pharmacologically. On the other hand, our lab reported that in the *Drosophila* system, NF1 null mutants showed olfactory learning defects, which can be remedied by overexpressing a constitutively active form of protein kinase A (PKA). An in-depth study of the Morris water maze paradigm indicated that the training protocol employed induced a form of memory that is protein-synthesis dependent (LTM). We hypothesized that the NF1 protein may be involved in different phases of learning and memory. By expressing fragments of the NF1 human transgene, as well as point mutations found in patients, to rescue learning and memory defects in NF1 null mutant flies, we found that NF1-GRD is necessary and sufficient to confer its functionality in protein-synthesis-dependent memory. In addition, our data from olfactory learning suggest that sequences in the carboxy-terminal region are able to rescue NF1's learning defects. In conclusion, we dissected the NF1 protein into regions of distinct functionality in the processes of the Pavlovian olfactory conditioning paradigm: The GTPase-activating protein (GAP)-related domain (GRD) is required for LTM, whereas the sequences in the carboxy-terminal region are essential for learning. These results suggest that NF1-dependent learning and memory processes are dependent on separate pathways.

A *Drosophila* Model of Alzheimer's Disease

K. Iijima, I. Hakker, H.-C. Chiang

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized clinically by memory impairments followed by global cognitive deficits. AD is defined by pathological changes in the brain, including neuronal loss, the formation of neurofibrillary tangles (NFTs), and senile plaques (SPs). AD is the most common senile dementia, and the number of AD patients is increasing as the world population ages. To date, there is no effective therapy for AD.

$A\beta_{42}$ is a major component of SPs and is derived from β-amyloid precursor protein (APP) by sequential cleavage of β-site APP-cleaving enzyme (BACE) and γ-secretase complex including presenilin1 or 2. More than 95% of AD cases are late-onset AD (LOAD) with-

out obvious familial segregation; however, there is a rare early-onset familial AD (EOFAD) with onset before age 65. To date, more than 160 mutations are found in three genes: *APP*, *presenilin1* (*PSEN1*), and *presenilin2* (*PSEN2*). All of these mutations enhance either production or aggregarion of $A\beta_{42}$ and accelerate its accumulation in the brain. Since there is no apparent difference in clinical and pathological phenotypes between LOAD and EOFAD, the accumulation of $A\beta_{42}$ in the brain during aging has been widely accepted as the causative event in AD pathogenesis. Extensive research efforts have been targeted on $A\beta_{42}$ to develop mechanism-based AD therapy. However, little is known about the molecular mechanisms underling memory defects and neurodegeneration caused by $A\beta_{42}$.

We use the fruit fly, *Drosophila*, as a model organism to study $A\beta_{42}$ toxicity in vivo. In addition to the state-of-the-art genetic tools, a number of behavioral tasks such as learning and memory enable us to monitor disease progression during aging. We have demonstrated for the first time that the transgenic *Drosophila*-expressing human $A\beta_{42}$ can faithfully reproduce AD-like phenotypes, including adult onset, progressive $A\beta_{42}$ amyloidosis, olfactory memory defects, and severe neuronal loss. This fly model is especially unique in terms of its extensive cell death, which is observed in AD patients but rarely seen in existing mouse AD models. Our results strongly support the idea that excessive accumulation of $A\beta_{42}$ is sufficient to cause memory defects and neuronal cell death and suggests that the molecular basis underlying the $A\beta_{42}$ toxicity can be conserved in a fruit fly.

To study the structure-toxicity relationship of $A\beta_{42}$ in vivo, we have established several new transgenic flies expressing mutated $A\beta_{42}$ with different aggregation/accumulation properties in the brain. Our fly model of AD will help us to understand the molecular and cellular mechanisms underlying memory defects and neurodegeneration induced by $A\beta_{42}$.

Imaging of Learning and Memory-associated Changes in the Mushroom Body in Adult *Drosophila* Brain

Y. Wang, A. Mamiya

The *Drosophila* mushroom body is a higher-order brain center that is crucial for olfactory learning and memory. It is a symmetrical structure, consisting of about 2500 intrinsic neurons in each brain hemisphere. There are three types of morphologically distinct mushroom body intrinsic neurons, also called Kenyon cells (KCs), in which axons are bundled separately into respective α/β, α'/β', and γ lobes. The development of the KCs follows a sequential order. Neurons that give rise to the γ lobe are born first, before the mid-third-instar larval stage, followed by those giving rise to α'/β' lobes, which are born between the mid-third-instar larval stage and puparium formation. Neurons that give rise to the α/β lobes are born last, after the puparium formation.

Studies have shown that the γ lobe is required for short-term memory. The α/α' lobes are suggested to be involved in long-term memory. However, how their involvement in learning and memory is manifested at the neuronal activity level is unclear. To address this question, we imaged neuronal activities in the mushroom body lobes with a genetically engineered calcium sensor after the flies were trained in a T maze where they learned to avoid an odor that is paired with electric shock to the feet. The rise in intracellular calcium is an indication of the activation of neurons. We found that activities in the α'/β' lobes in response to the shocked odor were much enhanced compared to an odor that was not paired with electric shock. However, little activities were observed in the γ lobe. One possibility for the lack of response in the γ lobe is that the calcium change in activated neurons is below the detection threshold of the calcium sensor.

Nevertheless, this finding is further confirmed with an isolated brain prep in which the olfactory T-maze training was mimicked by paired electrical stimulation of the antennal nerve (AN) and ventral nerve cord (VNC). Stimulation of the AN or VNC alone evoked activities in mushroom body lobes. A single pairing session of stimulation of the AN and VNC that mimicked a single olfactory training in behavioral studies resulted in enhanced activities in the α'/β' lobes in response to subsequent AN stimulation. The enhancement lasted up to 90 minutes in the β' lobe while much longer in the α' lobe. Unpaired stimulation of the AN and VNC did not induce any activity changes in the lobes in response to AN stimulation. We are currently assessing whether the enhancement of α'/β' activities can be correlated with memory phases revealed in behavioral studies.

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PLANT GENETICS

To casual observers, the astounding variety of the shapes, colors, and forms of plants lend great aesthetic beauty. But all of these traits—including those of tremendous agricultural significance—have been shaped by evolution or by human selection. Uncovering the molecular processes that govern how plants grow and develop is thus of fundamental biological interest and importance.

For example, the specialized shapes of leaves have a vital role throughout plant biology. Moreover, within each leaf, the functions of the upper and lower surfaces are very different. Marja Timmermans's work is focused in part on understanding how unspecialized stem cells create this dorsoventral (upper/lower) axis of leaves. Her group has identified two genes, *Leafbladeless1* (*Lbl1*) and *Rolled leaf1* (*Rld1*), that are essential for controlling this process. This year, Timmermans discovered that these genes are in turn controlled by the opposing action of two particular microRNAs. One significant aspect of these findings is that—like classical protein "morphogen gradients"—microRNAs can act as diffusible developmental signals that move through tissues and thereby control stem cell specialization.

Part of Rob Martienssen's recent work has also explored the role of microRNAs in plant development. In addition, this year, his group solved a century-old genetic puzzle concerning how the *RAMOSA1* gene controls the architecture of corn and other major cereal crops. The study also revealed telling information about the domestication of corn from its primitive ancestor.

Unlike developing animal embryos, plant embryos begin to differentiate dramatically from the very first cell division. This initial unequal cell division seals the fate of all subsequent tissue specializations in the developing embryo. Wolfgang Lukowitz studies the molecular steps that regulate this unequal cell division and its impact on later developmental events. His group has identified three proteins that are likely to form a signaling pathway that controls the earliest steps in plant embryonic development.

David Jackson and his colleagues have isolated a new gene involved in regulating branching in corn called *RAMOSA3*. They found that the gene encodes an enzyme that modifies the simple sugar trehalose. The discovery reveals a previously unknown role in plant development for this ubiquitous sugar. In other work, Jackson's group made significant progress in characterizing the structure and function of the tiny channels that link plant cells (called plasmodesmata) and which permit the long-range movement of proteins, RNAs, and plant viruses from cell to cell. By using a novel genetic screen to dissect the function of these channels, the researchers discovered the first known protein segment that is necessary and sufficient for cell-to-cell transport of molecular cargo through plasmodesmata. Finally, Jackson and his colleagues identified new clues about stem cell proliferation in plants (through their characterization of the *thick tassel dwarf* mutant) and "phyllotaxy" or the geometric arrangement of leaves (through their studies of the *ABPHYL1* gene).

CELL-FATE DECISIONS IN THE EARLY PLANT EMBRYO

W. Lukowitz M. Bayer, E. Glushenkova, S. Kotkin, T. Nawy, J. Nemiroff, S. Peters, K. Wetmore, J. Williams

In most plants, the early stages of embryogenesis follow a predictable sequence of cell divisions and cell-shape changes reflecting the coordinated fate decisions that lay down the reference points of the body plan. Our goal is to identify and understand the genetic network regulating this process. We are working with the small weed *Arabidopsis*, an inexpensive experimental model with very good comparability to economically important species.

A MAP Kinase Pathway Promoting Extraembryonic Fate

W. Lukowitz, M. Bayer, J. Williams

Plant development begins with an asymmetric division of the zygote. This division results in a fundamental fate decision that sets the stage for all subsequent patterning events: The small apical daughter cell will produce the embryo, whereas the large basal daughter cell will mainly form the extraembryonic suspensor. We are working to elucidate a signaling pathway that promotes suspensor fate in the basal cells.

The first component of this pathway to be identified was the MAPKK kinase gene *YDA*. Loss of *YDA* activity essentially eliminates formation of the extraembryonic suspensor, whereas hyperactive variants of *YDA* suppress formation of the embryo often to the extent that all daughters of the zygote appear suspensor-like. On the basis of these findings, we have proposed that the *YDA* MAP kinase cascade acts as a molecular switch that promotes extraembryonic or suspensor fate.

Recent unpublished results from the labs of J. Walker and S. Zhang (University of Columbia, Missouri) have identified a pair of MAP kinase genes: *MPK3* and *MPK6*, that act downstream from *YDA* in postembryonic development. In collaboration, we have confirmed that the same two MAP kinases also function in the embryo. The MAPK kinase genes of the *YDA* cascade have not yet been found. We are taking a systematic approach and are expressing hyperactive versions of all ten *Arabidopsis* MAPK kinase genes in the *yda*-deficient background to determine which ones can suppress the phenotype of mutant embryos.

Last year, we initiated the analysis of two other genes that encode key components of the *YDA* pathway, *SSP* and *GRD*. Both genes were originally identified in mutant screens: Mutations in *SSP* and *GRD* affect suspensor development in a similar way as loss of *YDA*. Double mutants of *yda* with *ssp* or *grd* do not have a more severe phenotype than *yda* single mutants. Furthermore, hyperactive versions of *YDA* can suppress the phenotype associated with *ssp* mutations, but have little effect on the phenotype associated with *grd* mutations. The simplest explanation for these observations is that *SSP* acts upstream of *YDA*, whereas *GRD* acts downstream from *YDA*.

The *GRD* gene encodes a RWP-RK protein. RWP-RK proteins constitute a small family of putative transcription factors that have only been found in green algae and higher plants. The first RWP-RK gene described in the literature was *minus dominance* (*mid*) from the unicellular alga *Chlamydomonas*. The *mid* gene dominantly determines the mating type of gametes. It is present in *mating-type*$^-$ cells, but absent in *mating-type*$^+$ cells. Introduction of *mid* into *mating-type*$^+$ cells is sufficient to make them differentiate as minus gametes. The second RWP-RK gene reported was *nodule inception* (*nin*) from the legume *Lotus japonicus*. The *nin* gene regulates a developmental fate decision triggered by the environment, namely, the symbiotic interaction between *Lotus* roots and *Mesorhizobium* bacteria that results in the formation of nitrogen-fixing nodules. One of the first steps in nodule formation involves signaling from the bacteria to the roots via a secreted lipochitosaccharide, termed Nod factor. The *nin* gene is thought to act downstream from the cell surface receptors for Nod factor to promote formation of infection threads and inception of nodules.

Interestingly, the *GRD* gene product contains two predicted MAP kinase phosphorylation sites, one of

which has a high score with different prediction algorithms and is conserved in the rice homolog of *GRD*. We have constructed variants of *GRD* in which one or both of these sites have been mutated such that they cannot be phosphorylated any more or such that they are mimicking the effects of constitutive phosphorylation. These variants will be functionally tested in mutant plants. If the predicted phosphorylation sites are required for normal *GRD* function in plants, we will investigate in vitro whether they are indeed modified by the *YDA* MAP kinase cascade.

SSP encodes a member of the receptor-like protein kinase family. In animals, these proteins operate in association with cell surface receptors, such as the Interferon-1 receptor complex. We have found that SSP protein is myristoylated at its amino terminus and that this modification effectively anchors the protein in the plasma membrane (Fig. 1). Myristoylation of SSP is essential for normal function: Mutation of the modified glycine residue causes mislocalization of the SSP protein to the cytoplasm where it remains inactive. Although SSP has been classified as a protein kinase, a key residue of the catalytic domain is missing and variants of SSP that harbor transition-state mutations in the ATP-binding pocket fully complement the phenotype of mutant embryos. This would suggest that SSP is a dead kinase. On the other hand, a small carboxy-terminal TRP domain of SSP that likely mediates protein–protein interactions is absolutely essential for normal function. Even deletions of a few amino acids create completely inactive variants. Taken together, our findings suggest that SSP functions as an adapter protein at the plasma membrane, perhaps as part of a receptor complex or as a link between a receptor complex and the *YDA* MAP kinase cascade.

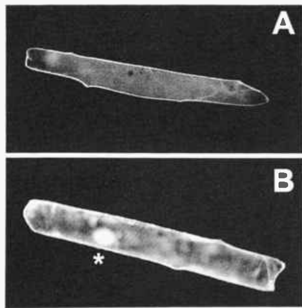

FIGURE 1 Localization of the *Arabidopsis* SSP protein. (*A*) SSP protein is anchored in the plasma membrane by the posttranslational addition of a myristoyl residue to its amino terminus. (*B*) Variants of SSP that cannot be modified are mislocalized to the cytoplasm and the nucleus (*star*) where they cannot function. The images show SSP protein that has been tagged with yellow fluorescent protein inserted between the myristoylation motif and the kinase domain. This fusion protein complements the phenotype of mutant embryos when expressed in plants. For the localization study, the fusion protein was transiently overexpressed in onion epidermal cells.

Our work so far can be summarized in a tentative outline of the signaling pathway regulating extraembryonic cell fate in *Arabidopsis*. At the center of the proposed pathway is the *YDA* MAP kinase cascade. This cascade appears to operate downstream from an as yet unknown cell surface receptor that is most likely triggered by an extracellular stimulus. The receptor-like cytoplasmic kinase *SSP* may form part of the receptor complex or act downstream from the receptor complex to activate *YDA*. Targets of the *YDA* MAP kinase cascade would include the transcription factors that execute suspensor-specific developmental programs, such as *GRD*.

We hope that a more complete analysis of the molecules involved in the *YDA* signal transduction pathway will eventually allow us to understand important aspects about the logic behind early plant development. For example, what is the nature of the signal that triggers the pathway, and where does it originate (perhaps in the endosperm surrounding the zygote or the maternal tissue of the seed coat)? How is down-regulation of the pathway in the apical cell and its daughters tied into the asymmetric division of the zygote?

Role of the *YDA* MAPKK Kinase Gene in Maintaining the Stem Cell Population of the Shoot Apical Meristem

W. Lukowitz, E. Glushenkova, S. Kotkin [in collaboration with G.V. Reddy and E.M. Meyerowitz, California Institute of Technology]

Plant growth is dependent on the continuous production of new organs, such as leaves, flowers, or lateral shoots. All of these organs are initiated by the apical meristems, a group of cells at the tip of the shoots. The apical meristems contain a small population of stem cells that are the ultimate progenitors of all organs.

The size of this stem cell population is regulated by a feedback loop between the stem cells themselves and the cells directly below them, called the organizing center. Stem cells secrete the small protein CLV3 that is perceived by a cell surface receptor complex including CLV1 and CLV2 and represses transcription of the homeodomain gene *WUS*. As a consequence, *WUS* mRNA is absent in the stem cells and only found in the cells of the organizing center (negative branch of the loop). The organizing center, in turn, provides an inductive signal required to maintain the stem cells in an undifferentiated state (positive branch of the loop). It has been proposed that a MAP kinase cascade mediates transduction of the CLV signal from the cell surface to the nucleus. However, experimental evidence for this claim is lacking, and no candidate genes have been reported that might function in such a cascade.

We have observed that seedlings harboring a hyperactive version of *YDA* often fail to form true leaves or do so with a substantial delay compared to wild type. This finding has prompted us to examine whether the MAPKK kinase *YDA* operates in the *CLA* signal transduction pathway. The shoot meristem of seedlings is located between the petioles of the two embryonic leaves or cotyledons. An analysis with the scanning electron microscope revealed that hyperactive variants of *YDA* frequently cause fusion of the cotyledons and eliminate the shoot meristem. Consistent with this result, CLV3 expression, which marks the stem cell population of the meristem, is undetectable in a large portion of embryos harboring hyperactive *YDA* variants (Fig. 2).

In collaboration with Venu Reddy in Elliot Meyerowitz's lab at Caltech, we are developing the tools to forcibly induce *YDA* activity in subsets of cells within the shoot apical meristem. The effect of these manipulations will be followed in live plants by imaging cell divisions and marker gene expression in the growing meristems. These experiments will enable us to test specific predictions derived from the premise that *YDA* operates in the *CLV* signaling pathway.

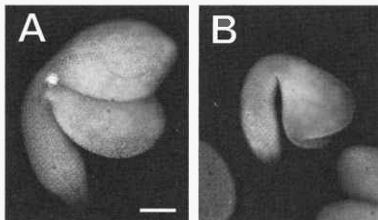

FIGURE 2 Hyperactive variants of the MAPKK kinase *YDA* eliminate the stem cell population of the shoot meristem. (*A*) In a mature wild-type embryo, the stem cells of the shoot meristem can be visualized with a reporter for *CLV3* expression (*white dot*). (*B*) No *CLV3* expression is detected in a seedling harboring a hyperactive variant of *YDA*, indicating that no stem cells are present. In addition, the cotyledons of this seedling appear to be fused. Images were taken with UV illumination, residual chlorophyll fluorescence outlining the embryos shown in gray and expression of the *CLV3*::green fluorescent protein reporter in white. Bar, 100 μm.

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PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen R. Carrasquillo, P. Ferreira, D. Garcia, D. Goto, J. Horn, D. Irvine, K. Izzo, A. Kloc, Z.B. Lippman, B. May, U. Ramu, G. Roche, D. Roh, M. Ronemus, R. Shen, J. Simorowski, R. Slotkin, M. Tanurdzic, M. Vaughn, E. Vollbrecht, M. Zaratiegui-Biurrun

Research in our laboratory concerns transposon silencing, gene control, and stem cell function in plants, as well as heterochromatic silencing in yeast, which provide useful models for higher organisms. In maize, stem cell function is restricted by the branching genes *ramosa1* and *ramosa2*, which have undergone differential selection during maize evolution and domestication. Stem cell function and axis formation in *Arabidopsis* depends on *asymmetric leaves1*, and on RNA interference (RNAi) via spatially restricted microRNA (miRNA) and *trans*-acting small interfering RNA (ta-siRNA). In fission yeast, RNAi of centromeric transcripts regulates histone modification, and we have found similar transcripts in *Arabidopsis*, where small RNA, DNA methylation, and chromatin remodeling regulate heterochromatin through transposons and repeats.

During the last year, Derek Goto left for a faculty position in Japan, Bruce May traveled to Vietnam to work in biotechnology, and Zach Lippman started a postdoctoral position in Israel, after graduating from the Watson School last year. We were joined by postdocs Danielle Irvine and Keith Slotkin, graduate student Anna Kloc, and URP Rob Carrasquillo. Paulo Ferreira joined the lab on sabbatical from the University of Rio de Janeiro.

Architecture of Floral Branch Systems in Maize and Related Grasses

E. Vollbrecht, R. Martienssen [in collaboration with E. Buckler, Cornell University]

The external appearance of flowering plants is determined by flower-bearing branch systems known as inflorescences. Inflorescence architecture reflects meristem number, arrangement, and activity, and the duration of meristem activity correlates with branch length. Unlike those of rice and sorghum, the inflorescences of maize mostly lack long branches, giving rise to the tassel and familiar corncob. The maize *ramosa1* gene controls inflorescence architecture via expression in a boundary domain near the nascent meristem base where it imposes short-branch identity. A second gene, *ramosa2*, acts through *ramosa1* by regulating gene expression levels. We have isolated *ramosa1* and *ramosa2* by transposon tagging, which has enabled identification of the respective genes. *ramosa1* encodes a zinc finger transcription factor that is absent in rice, heterochronically expressed in sorghum, and strongly selected during maize domestication and evolution. *ramosa2* is the maize ortholog of *LOB1*, an *Arabidopsis* gene detected by enhancer-trap expression at lateral organ boundaries. *LOB1* expression depends on *ASYMMETRIC LEAVES1* (*AS1*). *ramosa2* is also likely to encode a transcription factor, and is expressed in some of the same cells as *ramosa1*.

Specification of Leaf Polarity in *Arabidopsis* via *trans*-acting siRNA

D. Garcia, R. Martienssen [in collaboration with M. Byrne, John Innes Center]

In plants, leaves initiate on the flanks of the shoot apical meristem and subsequently develop distinct proximodistal, dorsoventral (adaxial-abaxial), and mediolateral patterns. The SANT (*myb*) domain gene *PHANTASTICA* (*PHAN*) is required for adaxial fate in the snapdragon *Antirrhinum* and in other plants, but the *Arabidopsis* ortholog *AS1* has milder effects on leaf shape, suggesting the existence of alternate or redundant regulatory functions. We performed a screen for enhancers of *as1* with more elongate and dissected leaves. These enhancers disrupt an RNA-dependent RNA polymerase (*RDR6*), *ARGONAUTE7* (*AGO7*)/*ZIPPY*, *SUPPRESSOR OF GENE SILENCING3* (*SGS3*), and *DICER-LIKE4* (*DCL4*), which all regulate ta-siRNA. Microarray analysis revealed that the *AUXIN RESPONSE FACTOR* genes *ETTIN* (*ETT*)/*ARF3* and

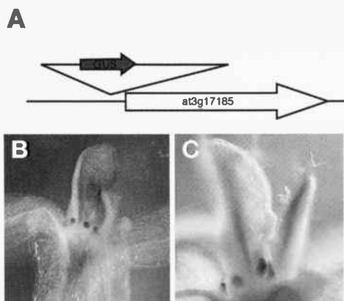

FIGURE 1 The *trans*-acting siRNA *TAS3* is a polarity gene. The gene-trap insertion GT19682 is located 37 bp upstream of the transcription start of the *TAS3* gene AT3g17185 (*A*) and results in GUS reporter gene expression (*dark*) in the upper, or adaxial, domain of emerging leaves (*B*,*C*). Strong staining is also observed in the stipules.

ARF4 were up-regulated in *ago7*, whereas *FILAMENTOUS FLOWER* (*FIL*) was up-regulated only in *as1 ago7* double mutants. *RDR6* and *SGS3* likewise repress *ARF3* and *ARF4*, which specify abaxial fate. We found that the *trans*-acting siRNA gene *TAS3*, which targets *ARF3* and *ARF4*, is expressed in the adaxial domain (Fig. 1) and that *ett as1 ago7* triple mutants resemble *as1*. Thus, *FIL* is down-regulated redundantly by *AS1* and by *TAS3*, acting through *ETT*, revealing a role for ta-siRNA in leaf polarity. *RDR6* and *DCL4* are also required for systemic silencing, implicating ta-siRNA as a potentially mobile signal. Interestingly, *TAS3* also matches one of six precursor genes for miR165/166, which we previously showed was abaxially expressed. Misexpression of miR166 in *TAS3* mutants could contribute to the phenotype in some cells, although miR166 target genes were still expressed in *as1 ago7* double mutants.

miRNA-targeted and siRNA-mediated mRNA Degradation Regulated by Argonaute, Dicer, and RdRP

M. Ronemus, M.W. Vaughn, R.A. Martienssen

ARGONAUTE1 of *Arabidopsis* mediates the cleavage of miRNA-targeted mRNAs, and it has also been implicated in the posttranscriptional silencing of transgenes and maintenance of chromatin structure. Mutations in *AGO1* severely disrupt plant development, indicating that miRNA function and possibly other aspects of RNAi are essential for maintaining normal patterns of gene expression. Using microarrays, we have found that 1–6% of genes display significant expression changes in several alleles of *ago1* at multiple time points, with the majority showing higher levels. Several classes of known miRNA targets increased markedly in *ago1*, whereas others showed little or no change. Cleavage of mRNAs within miRNA homologous sites was reduced but not abolished in an *ago1* null background, indicating that redundant slicer activity exists in *Arabidopsis*. siRNA (21–22 nucleotides), as well as larger (30–65 nucleotides) RNA fragments, corresponding to highly up-regulated miRNA target genes accumulated in wild-type plants, but not in *ago1*, nor in the RNA-dependent RNA polymerase mutants *rdr2* and *rdr6*, nor in the Dicer-like mutants *dcl1* and *dcl3*. Both sense and antisense RNAs corresponding to these miRNA targets accumulated in the *ago1* and *dcl1* backgrounds. These results indicate that a subset of endogenous mRNA targets of RNAi may be regulated through a mechanism of second-strand RNA synthesis and degradation initiated by or in addition to miRNA-mediated cleavage. siRNAs (21 nucleotides) corresponding to some targets (*TIR1*-like genes) were cleaved in register with the miRNA target site, resembling *trans*-acting siRNA.

RNA Polymerase II and the Slicer Function of Argonaute are Required for Heterochromatic Silencing and Spreading

D. Irvine, D. Goto, M. Zaratiegui-Biurrun, D. Roh, M. Vaughn, R. Martienssen [in collaboration with L. Joshua-Tor, Cold Spring Harbor Laboratory, and Y. Murakami, Kyoto, Japan]

In the fission yeast *Schizosaccharomyces pombe*, the RNAi machinery converts transcripts from pericentromeric repeats into siRNAs, which are required for the assembly of pericentromeric heterochromatin. A mutation in the second largest subunit of RNA polymerase II (Rpb2) converts an asparagine residue (conserved in *Arabidopsis*) into a tyrosine (conserved in budding yeast). Both wild-type and mutant Rpb2 localized to the pericentromere, and both were functional, but the mutation resulted in specific disturbance of pericentromeric heterochromatin caused by loss of histone modifications and accumulation of pericentromeric transcripts, accompanied by the loss of siRNAs. Thus, Rpb2 couples pericentromeric transcription with siRNA production. siRNA guides chro-

matin silencing via the Argonaute and RNA-dependent RNA polymerase complexes, and this silencing presumably depends on base pairing with either RNA or DNA. We have shown that Argonaute requires the conserved DDH amino acid motif for heterochromatic silencing and histone H3 lysine-9 dimethylation (H3K9me2). Argonaute proteins require this motif for RNase H activity, which cleaves (or slices) target messages complementary to siRNA. H3K9me2 spreads into silent reporter genes when they are embedded within heterochromatic transcripts. Silencing of these reporter genes requires readthrough transcription by polymerase II and processing of these transcripts via Argonaute. Thus, siRNA appears to impact histone modification by cleaving heterochromatic RNA.

Two Novel Proteins, Dos1 and Dos2, Interact with Rik1 to Regulate Heterochromatic RNAi and Histone Modification

D.B. Goto, M. Zaratiegui-Biurrun, R. Martienssen
[in collaboration with W. Zacheus Cande, University of California, Berkeley]

In fission yeast, the heterochromatin protein 1 homolog Swi6 recognizes H3K9me2, silences transcription, and retains cohesin at pericentromeric repeats. The DNA-damage-binding protein (DDB1) homolog, Rik1, and histone methyltransferase, Clr4, act in a complex to promote H3K9me2. We have shown that two novel genes, *dos1*$^{+}$ (*clr8*$^{+}$) and *dos2*$^{+}$ (*clr7*$^{+}$), are required for localization of Swi6. Deletion of either of these genes results in chromosome missegregation, defects in mitotic centromeric cohesion, meiotic telomere clustering, and loss of heterochromatic silencing. Dos1 is predominantly located in the nucleus in a Dos2-dependent manner and directly interacts with Rik1. Each of these genes is required for the association of H3K9me2 with centromeric repeats, as well as for the production of siRNA. Rik1 has a WD-repeat CPSF domain found in RNA as well as DNA-binding proteins.

Differential Regulation of Strand-specific Transcripts from *Arabidopsis* Centromeric Satellite Repeats

B. May, Z.B. Lippman, R. Martienssen [in collaboration with D. Spector, Cold Spring Harbor Laboratory]

Centromeres interact with the spindle apparatus to enable chromosome disjunction, and typically contain thousands of tandemly arranged satellite repeats interspersed with retrotransposons. Although their role has been obscure, centromeric repeats are epigenetically modified, and centromere specification has a strong epigenetic component. In *S. pombe*, long heterochromatic repeats are transcribed and contribute to centromere function via RNAi. In *Arabidopsis*, as in mammalian cells, centromeric satellite repeats are short (180 bp), found in thousands of tandem copies, and they are methylated. We have found transcripts from both strands of canonical, bulk *Arabidopsis* repeats. At least one subfamily of 180-bp repeats is transcribed from only one strand and regulated by RNAi and histone modification. A second subfamily of repeats is also silenced, but silencing is lost on both strands in mutants in the CpG DNA methyltransferase MET1, the histone deacetylase HDA6/SIL1, or in the chromatin remodeling ATPase DDM1. This regulation is due to transcription from Athila2 retrotransposons, which integrate in both orientations relative to the repeats, and differs between strains of *Arabidopsis*. Silencing lost in *met1* or *hda6* mutants is reestablished in backcrosses to wild type, but silencing lost in RNAi mutants and in *ddm1* is not. siRNA (24 nucleotides) from centromeric repeats are retained in *met1* and *hda6*, but not in *ddm1*, and may have a role in this epigenetic inheritance. Histone H3 lysine-9 dimethylation (H3K9me2) is associated with both classes of repeats.

Polyploidy, Transposable Elements, and Epigenetic Control

M. Tanurdzic, Z.B. Lippman, R. Carrasquillo, K. Slotkin, M. Vaughn, R. Martienssen [in collaboration with P. Rabinowicz, TIGR; L. Comai, University of Washington; W. Thompson, N. Carolina State University; and R.W. Doerge, Purdue University]

Heterochromatin is composed of transposable elements (TE) and related repeats. Like TEs, heterochromatin silences genes located nearby and has a major role in epigenetic regulation of the genome. siRNA corresponding to heterochromatic sequences can be detected in plants, animals, and fission yeast, indicating that these sequences are transcribed. We have used tiling microarrays to examine these transcripts and their regulation. In plants, siRNA corresponding to different classes of TE depends on the DNA methyltransferase MET1, the SWI/SNF ATPase DDM1, or both, but not on the histone deacetylase SIL1. All three genes are required for silencing transposons in the absence of RNAi, but they depend on siRNA for resilencing in backcrosses. DNA methylation is concentrated in TEs

and heterochromatin, but many genes have low levels of methylation in their coding region. Genic methylation is not associated with H3K9me2, is highly polymorphic, does require DDM1, and is inherited in recombinant inbred lines, but it has little effect on gene expression.

Polyploidy in plants results in a variety of genetic and epigenetic changes in gene expression from generation to generation following the establishment of allopolyploids. The mechanisms that underlie these changes are important in the short term (hybrid vigor) as well as in the long term, as polyploids are stabilized during evolution. We are profiling chromatin modifications that may underlie novel gene expression in synthetic allopolyploids derived from crosses between tetraploid *A. thaliana* and tetraploid *A. arenosa*, as well as those derived from crosses between doubled haploids of *Brassica oleracea* and *B. rapa*. Although changes in heterochromatin are relatively modest, some transposons are activated in the allotetraploids. We are also comparing diploid chromatin modification profiles with the pattern of replication of chromosomal DNA using BrdU incorporation in synchronized cells.

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PLANT SIGNALING AND DEVELOPMENT

D. Jackson Y. Benitez, P. Bommert, K. Lau, B.-h. Lee, A. Leibfried, J. Linder, S.J. Locke, A. Maizel, A. Mohanty, N. Satoh-Nagasawa, N. Seidl, J. Wang, Y. Yang, P. Yin

Plants display incredible morphological diversity, making them a rich system for studies in developmental biology. Our lab is working to identify the molecular signals that regulate pattern formation and morphogenesis during plant growth. These signals regulate stem-cell activity, cell division, and expansion and have shaped plant evolution and crop development. In the past year, we have made significant progress in our studies of intercellular transport of regulatory proteins through tiny channels called plasmodesmata. We have used a novel genetic screen to dissect the function of these channels and have reported the first known domain in any protein that is both necessary and sufficient for cell-to-cell transport. We also reported a new player in the classical *CLAVATA* signaling pathway in maize. The *thick tassel dwarf* mutant encodes a receptor kinase whose expression regulates stem-cell proliferation, and genetic evidence suggests that it may act independently of *FASCIATED EAR2*, a receptor-like protein that we previously characterized. Biochemical experiments to investigate possible interactions between these signaling proteins are under way. We also continue our studies of the *ABPHYL1* gene. Mutations in this gene are unique in that they alter the geometric arrangement of leaves made by the shoot, and this gene encodes a protein that regulates signaling by the hormone cytokinin. Our recent results suggest an intriguing and possibly direct interaction of this gene with auxin signaling and transport.

We have also isolated the *RAMOSA3* gene, which is involved in regulating inflorescence branching in maize. *RA3* encodes a phosphatase, and we found that the substrate of this enzyme is a simple disaccharide, trehalose. Our results point to a novel developmental role for this ubiquitous sugar. Our ultimate goal is to link the different signaling pathways into networks of regulatory activities, giving rise to a "virtual" plant. To this end, we have embarked on genomics-enabled projects to characterize the localization and interaction partners of developmental proteins in maize and rice. These plants are model systems as well as important crops, and our findings point to important differences in regulatory networks between these "monocot" plants and the more common model, *Arabidopsis*.

Studies of Maize Phyllotaxy

B.-h. Lee, Y. Yang, A. Mohanty

We are interested in how leaves are geometrically arranged in plants—a fascinating phenomenon called phyllotaxy. We have previously reported a characterization of the maize *abphyl1* (*abph1*) mutants and cloning of the *ABPH1* gene. *abph1* mutants have opposite pairing of leaves, instead of the normal alternating pattern, and the *ABPH1* gene encodes a cytokinin-inducible type A response regulator. In the past year, we continued to investigate the function of *ABPH1* in phyllotaxy regulation.

As many genes involved in cytokinin signaling have been identified in *Arabidopsis*, a dicot model plant, the developmental phenotypes of *Arabidopsis* mutants defective in several cytokinin signaling genes were examined; however, no distinct phyllotactic defects were observed, suggesting that the maize *abph1* mutants provide a unique genetic system to study phyllotaxy.

Another plant hormone, auxin, has been implicated in phyllotaxy regulation, and thus we studied whether *ABPH1* may interact with auxin signal transduction. First, we localized *ABPH1* expression in shoot apices cultured with an auxin polar transport inhibitor (*N*-1-naphthylphthalamic acid [NPA]) and found that this treatment represses *ABPH1* expression in the shoot apical meristem. This result suggests that polar auxin transport is required for *ABPH1* expression. A second approach was immunolocalization of PINFORMED1 (PIN1) in the maize shoot apex using an *Arabidopsis* anti-PIN1 antibody. PIN1 protein is a polar auxin efflux facilitator and is involved in phyllotaxy regulation. We found that the *Arabidopsis* anti-PIN1 antibody can detect the maize PIN1 protein (ZmPIN1) in the same pattern found in *Arabidopsis*, in

a polar localization on one side of the cell. In addition, we found that ZmPIN1 localization marks maize leaf initiation in a manner similar to that in *Arabidopsis*. ZmPIN1 expression appeared to be lower in *abph1* than in normal maize meristems. Quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) assays also suggest a reduced expression of *ZmPIN1* in *abph1* mutants. These results indicate that *ZmPIN1* expression is dependent on *ABPH1* and a mutual interaction exists between auxin and cytokinin-regulated phyllotaxy control.

Type-A response regulators in *Arabidopsis* can function as either activators or repressors in cytokinin signaling. Our genetic data suggest that *ABPH1* acts as a repressor. To investigate this, we overexpressed the rice *ABPH1* homolog in rice callus tissue that was induced to regenerate. We observed a failure of shoot regeneration in the *ABPH1*-overexpressing calli, confirming that *ABPH1* acts as a repressor of shoot development. We also produced transgenic maize lines harboring the *ABPH1* gene internally fused with yellow fluorescent protein under the control of the *ABPH1* endogenous promoter. This transgene was made using the "fluorescent tagging of full-length proteins" method developed in our lab. Transient expression of the fusion in onion epidermal cells revealed nuclear localization of the ABPH1 fusion protein. In maize plants, ABPH1-YFP was specifically expressed in shoot apical meristems, in a pattern similar to that previously observed by in situ hybridization. These *ABPH1-YFP*-expressing plants will facilitate *ABPH1* expression studies to understand its role in phyllotaxy regulation.

We have also carried out microarray analysis of normal versus *abph1* embryos collected 10 days after pollination, when the shoot apical meristem is just initiated. For robust statistical analysis, we included five biological replicates. The expression of 377 genes is affected in *abph1* mutants, with 314 genes expressed lower and 63 genes higher in *abph1*. Some of these genes include auxin response factors, a putative auxin transporter-associated protein, and a protein involved in auxin conjugation, supporting the interaction between *ABPH1* and auxin signaling pathways. These results will be validated and candidate target genes studied using a reverse genetics strategy.

To identify additional factors involved in the control of phyllotaxy, we identified a second maize phyllotaxy mutant, *abph2*. Like *abph1*, the *abph2* mutant has a bigger shoot meristem than normal and shows abnormal phyllotaxy. We mapped *ABPH2* to the short arm of chromosome 7 using molecular markers. To facilitate positional cloning of *ABPH2*, transposon tagging and ethylmethane sulfonate (EMS)-mutagenized mutant populations are being generated and will be screened for additional alleles.

Characterization of the *RAMOSA3* Gene of Maize

N. Satoh-Nagasawa, J. Linder [in collaboration with N. Nagasawa, H. Sakai, DuPont; S. Malcomber, California State University, Long Beach; D. Johnson, University of Illinois; W. Huang and V. Brendel, Iowa State University]

Inflorescence branching is one of the major yield traits in crop plants, and it is controlled by the developmental fate of axillary shoot meristems. To obtain insight into the genetic and molecular mechanism of inflorescence branching, we characterized the *ramosa3* (*ra3*) mutant, which has enhanced inflorescence branching. Scanning electron microscopy showed that *RA3* functions to establish the correct identity and determinacy of axillary meristems in the maize inflorescences. We cloned *RA3* by positional cloning, and it encodes a predicted protein with significant similarity to trehalose-6-phosphate phosphatases (TPPs) and is expressed predominantly in young inflorescences in a localized pattern at the base of the axillary meristems (Fig. 1).

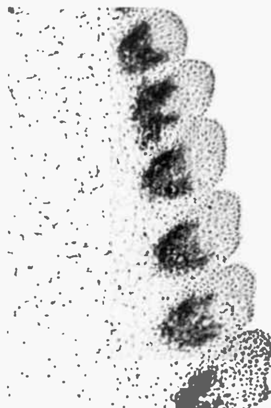

FIGURE 1 In situ hybridization in developing maize ear primordia, showing expression of *RAMOSA3*, at the base of the axillary meristems.

For further characterization of *RA3*, we took several approaches. To investigate whether *RA3* has TPP activity, we performed phosphatase assays and yeast complementation tests. Both experiments suggested that *RA3* acts specifically as a TPP in vitro as well as in vivo. To investigate whether *RA3*-like genes might also have a developmental function in other plants, we isolated homologs from several grasses. Phylogenetic and expression analyses indicate that *RA3* function is probably conserved at least throughout the grasses. To elucidate the genetic pathway in which *RA3* acts, we also compared global gene expression in wild-type and *ra3* mutant developing ears by microarray analysis. We identified some candidate developmental genes with statistically significantly different expression levels, and these are being verified by quantitative RT-PCR. In a candidate gene approach to find other players in the *RA3* pathway, we analyzed double mutants and examined the expression of *RA3* and other genes in single and double mutants. Surprisingly, we found that *RA3* functions upstream of the predicted transcriptional regulator *RAMOSA1*, suggesting that *RA3* may have a transcriptional role in addition to its enzymatic function.

Our studies provide the first indication that trehalose metabolism can regulate specific developmental pathways, and the nature of *RA3* is novel among genes that regulate maize inflorescence branching. Thus, we expect to obtain unique insight into the connection between sugar metabolism and maize inflorescence development by further investigation of *RA3*.

Molecular and Genetic Characterization of Plant Intercellular Channels

Y. Benitez, J. Wang, P. Yin, S.J. Locke

Communication between cells regulates development in both animals and plants. In plants, this function involves plasmodesmata (PD) channels that traverse the cell walls of neighboring plant cells. PD not only provide cell-cell connectivity but, more importantly, function to exchange metabolites and nucleic acids and protein signals. Despite their importance, PD structure and function are far from resolved. Our group invests great effort toward this goal through the characterization of *Arabidopsis* mutants that show a defect in PD trafficking.

We have isolated a number of mutants that exhibit a restriction in the unloading of green fluorescent protein (GFP) from the phloem vascular cells of the root to the surrounding meristematic cells. These mutants were named *gat* (pronounced "gate") for *G*FP *a*ltered *t*rafficking. We are currently focused on the characterization of two of these mutants, *gat1* and *gat2*. We have determined that both are recessive and show a seedling-lethal phenotype. Our immediate aims are to determine which genes are mutated and to identify the specific trafficking pathways disrupted in these mutants.

Both *gat1* and *gat2* mutants appear to be morphologically normal during the globular and heart stages but are delayed in their development. The major difference is seen when embryos reach the cotyledon stage. For example, *gat2* mutants have a large embryo structure that seems unable to bend within the seed coat. Symplasmic trafficking is being analyzed in mutant embryos using dye-loading experiments. As expected, wild-type embryos traffic HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid), a 0.5-kD dye, in all embryonic stages (Fig. 2). We are studying the transport of this and larger dyes such as a 10-kD F-dextran-fluorescein isothiocyanate conjugate to compare their movements in wild-type and *gat* embryos.

gat1 plants were also crossed with cell-specific GFP lines, obtained from the Benfey lab of Duke University, and used to confirm that mutant embryos and seedlings form normal phloem and root meristem structure. By quantification of GFP restriction in *gat1* seedlings expressing cytoplasmic GFP and in *gat1* seedlings expressing GFP-ER, we believe that *gat1* is affected in the GFP unloading out of the companion cells (CC) of the phloem. We are also interested in testing the systemic propagation of posttranscriptional

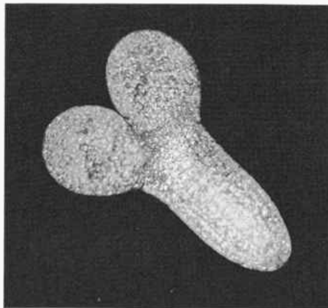

FIGURE 2 *gat2* mutant embryos loaded with HPTS show symplasmic connectivity (fluorescence) throughout embryonic tissues.

gene silencing/RNA interference (RNAi), using lines provided by the Voinnet lab of CNRS (Strasbourg, France). Our preliminary results suggest that *gat1* is not affected in spreading of the RNAi signal from the phloem to surrounding tissues, and we are currently testing other *gat* mutants. To analyze the targeted trafficking of developmental proteins, such as SHR and STM, in *gat* mutant meristems, we are crossing them to GFP reporter lines for these proteins. Finally, we are starting to study morphological differences in PD assembly and structure using transmission electron microscopy (TEM) with the help of Stephen Hearn here at CSHL.

The region containing the *gat1* mutation has been narrowed down to a 22-kb region of chromosome 2 using fine mapping. We are currently conducting complementation analyses with candidate genes to determine which one is responsible for the observed phenotype. T-DNA insertion lines of the putative mutated genes have also been obtained, and we are performing allelism tests. Similarly, we have made progress in the mapping of *gat2*. We have fine-mapped the mutation to an approximately 35-kb region of chromosome 1 that contains nine genes. Candidate genes are being sequenced from the mutant, and allelism tests with other embryonic-lethal mutants located in this genomic region are being conducted to identify new alleles.

Toward an Understanding of the Role of mRNA Trafficking by Transcription Factors in Plants

A. Maizel [in collaboration with ISV, CNRS Gif-sur-Yvette, France]

The intercellular transfer of regulatory transcription factors and mRNAs has become a new paradigm in plant biology in recent years. The transcription factor KNOTTED1 (KN1) was the first such example of a developmental signal that traffics from cell to cell. Its conserved DNA-binding domain, the homeodomain, is sufficient to mediate its own transfer from cell to cell in vivo, as well as promoting transfer of the KN1 mRNA (Kim et al. 2005).

RNA-binding activity of transcription factors has already been reported. The best-studied example is the homeodomain protein BICOID, which binds *caudal* mRNA and represses its translation in the *Drosophila* embryo. The homeodomain is sufficient to bind directly to both RNA and DNA molecules, and a specific position of the homeodomain DNA recognition helix discriminates RNA versus DNA binding. However, noncell autonomous activity of *bicoid* has never been reported, which makes the observation that KN1 can escort its own messenger more intriguing and raises questions about the molecular mechanism and the significance of this transfer. A recent project developed in the lab aims at understanding the molecular mechanisms as well as, in the long term, the physiological significance of an interaction between a trafficking transcription factor and its mRNA.

As the homeodomain of KN1 is necessary and sufficient to traffic from cell to cell and mediate transfer of the KN1 mRNA, we postulated that a direct interaction between the homeodomain and the RNA is likely. To gain further insights in the molecular mechanisms of this interaction, we tested in yeast the interactions between KN1 and its mRNA. We used the yeast 3-hybrid system, which allows for detection of RNA/protein interaction in a cellular context. Not knowing which part of the messenger is required for the interaction, we used a systematic approach by testing overlapping fragments covering the whole KN1 mRNA. Preliminary results indicate weak interactions between KN1 and two fragments of its mRNA that have stem-loop conformations. Further in vitro experiments are currently under way to confirm this interaction.

To address the question of where in the cell this interaction takes place, we adapted to plants an innovative system to detect protein/RNA interaction in vivo. A portion of the Venus fluorescent protein (Venus FP) will be tethered to the KN1 mRNA by the well-characterized bacteriophage MS2 coat protein–RNA operator interaction. The complementary portion of Venus FP will be fused to the KN1-HD. If the KN1-HD is able to associate with KN1 mRNA, it will bring the two portions of Venus FP in close proximity to form a fluorescent complex, while simultaneously identifying their site of interaction within the cell. Recombinant clones have been obtained and the first assays are currently under way.

Regulation of Meristem Size in Maize

P. Bommert, K. Lau, N. Seidl, A. Leibfried, J. Wang
[in collaboration with W. Bruce, Pioneer]

Shoot meristems have the remarkable ability to regulate their size during development. They do this by balancing stem-cell division with the incorporation of cells into new primordia. In *Arabidopsis*, this balance is controlled by the *CLAVATA* signaling pathway. The current

model proposes that a heterodimeric receptor complex composed of the receptor kinase *CLAVATA1* and the receptor-like protein *CLAVATA2* limits the expression of the stem-cell-promoting factor *WUSCHEL*. Analysis of the *fasciated ear2* (*fea2*) and *thick tassel dwarf1* (*td1*) genes in maize suggests that this pathway is conserved among angiosperms, as *fea2* encodes an a leucine-rich repeat (LRR) receptor protein orthologous to *CLV2* and *td1* encodes an LRR receptor-like kinase orthologous to *CLV1*. We are presently using biochemical approaches to characterize the FEA2 receptor complex. The size of the FEA2 complex has been determined to be 450 kD, using gel-filtration chromatography and glycerol gradient centrifugation. We hope to identify other components of this complex by analyzing its size in extracts from other fasciated mutants.

We have also constructed a genomic FEA2 fusion to the tandem affinity purification (TAP) tag, using the endogenous *fea2* promoter. Initial immunoblot analysis indicates that the FEA2-TAP fusion protein is expressed at levels equal to, or higher than, the endogenous FEA2 protein. Currently, we are in the process of purifying the FEA2 complex using the TAP epitopes and will perform mass spectrometric analysis of the complex to identify novel components. Any interactors that are identified will be analyzed by expression profiles, mutant phenotypes, and genetic interactions with *fea2* and *td1*.

We have also started to isolate the *compact plant2* (*ct2*) gene via mapped based cloning. *ct2* mutants develop strongly fasciated ears, indicating that this mutation affects meristem size regulation similar to *fea2* and *td1*. The *ct2* gene was previously mapped to the short arm of chromosome 1. By using a combination of SSR and CAPS markers and 150 mutant individuals, we were able to locate the chromosomal position of *ct2* to a 3-cM interval. Fine mapping of the *ct2* locus using approximately 1000 mutants is in progress, and we have isolated several new alleles of *ct2* that will enable identification of the gene once we have mapped it to a small interval.

Developing Tools for a Protein–Protein Interaction Map of Rice

A. Mohanty [in collaboration with M. Myers and W.R. McCombie, Cold Spring Harbor Laboratory]

We have developed a high-throughput methodology to tag plant genes in their native genomic context for functional analysis, including their subcellular localization and identification of interacting proteins. Recently, we have extended this approach, originally developed for *Arabidopsis*, to the economically important crops rice and maize. Although the rice genome has been fully sequenced, the function of only a handful of genes is known. To fill this gap, we are using a plant-optimized TAP tag with the aim of studying protein–protein interactions. We have already generated several transgenic rice lines harboring TAP-tagged rice proteins expressed under their native promoters, and protein complexes isolated from these lines are being analyzed by mass spectroscopy to identify the interacting proteins. We would like to extend this work to generate a comprehensive map of interacting proteins in rice.

Hormonal Signaling in Maize

A. Mohanty, Y. Yang [in collaboration with A. Chan, The Institute of Genomic Research; X. Ling and A. Sylvester, University of Wyoming]

Plant hormones have critical roles in growth and development. Although they have been studied for decades, their molecular mechanism of action is poorly understood. Recently, there has been significant progress, as receptors for ethylene, auxin, and cytokinins have been identified. Among these, cytokinins are especially interesting as they regulate a variety of processes including crop yield. Cytokinins are perceived by histidine kinase receptors (HKs), which upon binding of cytokinins initiate a phosphorelay transfer to response regulators (RRs) via histidine phosphotransfer (HP) proteins. The B-type response regulators act as transcription factors to activate the A-type response regulators and regulate a variety of downstream cellular processes. We recently uncovered a specific developmental role for the maize A-class RR gene *ABPHYL1*, in the regulation of meristem size and phyllotaxy.

We have taken a localization-based approach to decipher the developmental roles of the auxin and cytokinin signaling pathways in maize. We are generating genomic fusions with fluorescent proteins to the HPs, HKs, and RRs, as well as cytokinin oxidase and auxin transporter genes, and are generating maize plants expressing these fusions. We are interested in addressing whether the subcellular localization of these proteins is regulated in a tissue-specific manner and whether the nucleocytoplasmic shuttling observed in transient assays holds true for these genes expressed in their native developmental context. We are also

interested in evaluating expression patterns in multiple organs and cell types to develop an atlas of expression patterns in maize. These lines will greatly aid our understanding of plant hormone signaling. Since the subcellular and tissue-specific localization signals have hardly been characterized in maize, we are also generating a series of marker lines for compartments such as the nucleus, cytoskeleton, tonoplast, and peroxisome to aid data interpretation. We have already obtained maize lines harboring some of these markers, and they are being analyzed by confocal microscopy. In the coming year, we will continue our expansion into using proteomics, microarrays and genome-scale localization approaches to piece together developmental signaling pathways into integrated networks.

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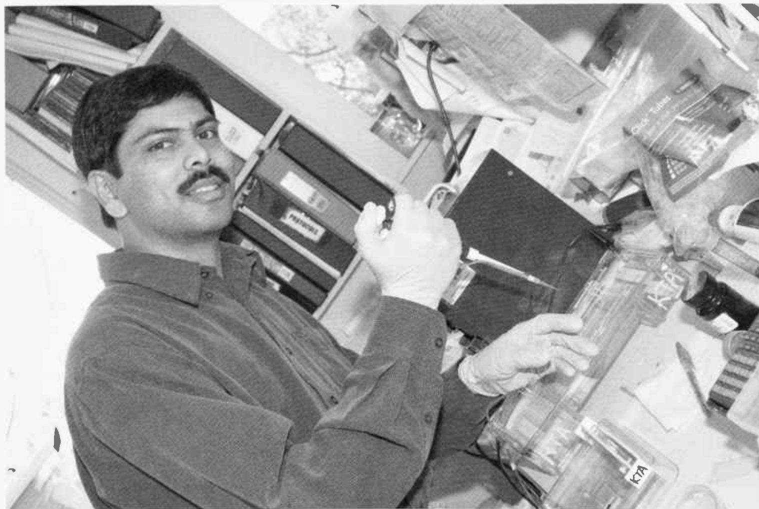

Amitabh Mohanty

PLANT DEVELOPMENTAL GENETICS

M. Timmermans M. Guo F. Nogueira
S. Madi T. Phelps-Durr
K. Marran

Development in higher plants is a continuous process as organs emerge throughout the entire plant life cycle, which for some plants extends over hundreds of years. The growing tip of a plant, referred to as shoot apical meristem (SAM), contains a population of stem cells that divide to maintain the SAM and to generate daughter cells from which lateral organs, such as leaves and flowers, arise. The research in our lab aims to understand the molecular mechanisms that distinguish indeterminate stem cells from their differentiating derivatives. In addition, we are studying the role of stem-cell-derived signals in the patterning of lateral organs. Observations from our lab indicate that microRNAs (miRNAs) are among these meristem-derived signals. Moreover, stem cells produce signals required for the establishment of determinacy. This process involves an epigenetic silencing mechanism, indicating that the switch from stem cell to differentiated cell is not simply encoded in DNA but also by proteins associated with DNA.

Adaxial/Abaxial Patterning of Lateral Organs in Maize

F. Nogueira, S. Madi

Outgrowth and patterning of the leaf requires the establishment of adaxial/abaxial (dorsoventral) polarity. Last year, we reported that this asymmetry is specified in part through the polarized expression of class III homeodomain leucine zipper (HD-ZIPIII) transcription factors that specify adaxial/upper fate. Through in situ hybridization analyses we were able to show that the adaxial specific expression of the *hd-zipIII* gene *rolled leaf1* (*rld1*) is set up by the expression pattern of a 21-nucleotide miRNA, miR166. miRNAs are endogenous, small (~22 mer), noncoding RNAs that mediate the cleavage or translational repression of target transcripts containing a complementary sequence, and thus control gene expression posttranscriptionally. Interestingly, we showed that the peak of miR166 expression occurs immediately below the incipient leaf and below the domain in which miR166 acts on the *hd-zipIII* genes, but that an expression gradient of miR166 is established that spreads from the abaxial/lower site throughout the developing leaf and sets up leaf polarity.

Specification of adaxial fate also requires the activity of *leafbladeless1* (*lbl1*). Recessive mutations in *lbl1* lead to formation of abaxialized leaves. Double mutant and expression analyses indicate that *lbl1* acts upstream of *rld1* and is required for the accumulation of *rld1* transcripts in the developing leaf. The effect of *lbl1* on polarity is mediated via miR166. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis showed that transcript levels for *mir166c*, *mir166h*, and *mir166i* are increased in *lbl1* apices compared to wild type. Moreover, using in situ hybridization we have shown that loss of *lbl1* function affects the pattern of miR166 accumulation and leads to misexpression of miR166 throughout the initiating leaf. These results indicate a key role for *lbl1* in the spatiotemporal regulation of miR166.

We have cloned *lbl1* and found that this gene encodes SUPPRESSOR-OF-GENE SILENCING3, which is required for the biogenesis pathway of a second family of small regulatory RNA, the *trans*-acting short interfering (ta-siRNAs). We have identified four loci in maize that generate ta-siRNAs. These *tas* loci produce typical RNA-PolII transcripts that are capped and polyadenylated. Importantly, the *tas* transcripts are targets for miR390-directed cleavage. However, in contrast to most miRNA-directed cleavage products, the resulting *tas* cleavage fragments are converted to double-stranded RNAs (dsRNAs) and are subsequently processed by Dicer into 21-bp ta-siRNAs. Because Dicer cleavage initiates at the processed end of the *tas* precursor, ta-siRNAs are generated in a 21-nucleotide phase starting at the miR390 cleavage site. The ta-siRNAs generated from each *tas* locus can therefore be predicted, and this enables the identification of potential target genes using computational approaches. Interestingly, we have found that the primary *mir166i* transcript has complementarity to one of the ta-siRNAs: ta-siR2142. This suggests that this *mir166* precursor could be a direct ta-siRNA target, and we are currently using 5′ RACE analysis to test if the effect of

lbl1 on *mir166i* expression is mediated *directly* by ta-siR2142. In addition, ta-siR2142 regulates expression of *auxin response factor3* (*arf3*) genes. However, the *mir166c* and *mir166h* precursors have no complementarity to the known ta-siRNAs, indicating that the effect of *lbl1* on their expression is indirect. The possibility that expression of these *mir166* precursors is under control of the *arf3* genes is being investigated.

Our observations indicate that SGS3 is essential for adaxial/abaxial patterning in maize, and we present the intriguing possibility that adaxial/abaxial polarity is specified through the opposing action of two small regulatory RNAs: ta-siR2142 and miR166. Our results further illustrate the complexity that can be found in small RNA-controlled networks, because we have shown that the expression pattern of miR166 is under control of ta-siR2142, which in turn is regulated by miR390. By studying the genetic pathway involving *lbl1* and *rld1*, we hope to improve our understanding of the role of small regulatory RNAs as developmental signals.

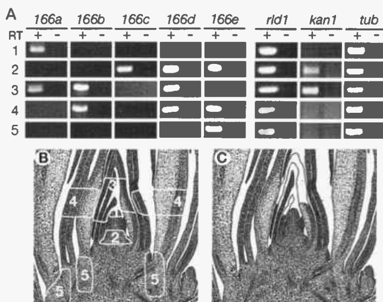

FIGURE 1 *mir166* genes are expressed in distinct but overlapping domains within the vegetative apex. (*A*) Cells were captured from five regions within the vegetative apex: 1, tip of the SAM; 2, base of the SAM; 3, young leaf primordia (P2, P3); 4, older leaf primordia (P4, P5); 5, stem tissue. mRNA was linearly amplified and used in one-step RT-PCR to monitor expression of selected *mir166* genes as indicated. Loading and RT controls are shown. (*B*) Longitudinal section of a maize apex with a diagram of the regions captured by LCM. (*C*) Longitudinal section of an apex after capturing cells from the young leaf primordia (P2, P3). Note the precision with which cells can be captured.

The Spatiotemporal Regulation of miR166 Expression in Developing Leaves

F. Nogueira

This work was performed with contributions from D. Chitwood, WSBS rotation student.

The dynamic expression pattern and the gradient of miR166 expression are reminiscent of a movable signal and suggest that expression of the *mir166* precursors is under such control or, alternatively, that miR166 can move between cells. Obviously, resolving these possibilities is of key importance because the knowledge that miRNAs can act as mobile signals will have major implications regarding the role of small RNAs in developmental biology. The maize genome contains at least nine *mir166* genes, suggesting that the dynamic miR166 expression pattern may result in part from the differential regulation of individual *mir166* family members. Expression analyses of individual *mir* genes by in situ hybridization have been unsuccessful. We therefore used laser capture microdissection (LCM) in combination with RT-PCR to analyze the expression profiles of *mir166* genes in specific domains of the SAM and young leaves (Fig. 1). Of the *mir166* genes we have tested thus far, each display unique tissue and cell-type-specific expression patterns. Our results indicate that the pattern of miR166 accumulation likely results in part from the intricate transcriptional regulation of its precursors. One of the tissue samples tested included cells from immediately below the incipient leaf that accumulates relatively high levels of miR166. Three *mir166* family members are expressed in that domain and are thus important for setting up adaxial/abaxial leaf polarity. Interestingly, the *mir166a* precursor was found to be expressed at the tip of the SAM. This result was surprising, because these cells do not accumulate miR166 and express the target *rld1*. This suggests that miR166 accumulation may be regulated in part at the posttranscriptional level. At this time, LCM RT-PCR analysis on more precisely defined developmental domains in the SAM and leaf primordia is being used to establish whether miR166 is mobile.

We have shown that in *Arabidopsis* miR166 exhibits a similar highly dynamic expression pattern. Four *MIR166* family members are expressed in vegetative tissues, including *MIR166a*. The 5′ end and polyadenylation sites of the *MIR166a* primary transcript were identified, and we found that *MIR166a* uses two distinct transcription start sites. Using *promoter::GUS* transcriptional fusions, we established the expression pattern of *MIR166a* in developing

seedlings. *MIR166a* is expressed in the apical region, but not in young leaves. In older leaves, expression initiates at the tip and extends basipetally in both vascular and ground tissues. Promoter deletions showed that a 200-bp promoter fragment is sufficient for vascular expression. Interestingly, this small fragment is part of the larger *MIR166a* primary transcript, providing another example of the complex regulation of miR166 expression. Several observations suggest that expression of *MIR166a* may be regulated by the plant hormone auxin. Its promoter includes binding sites for auxin response transcription factors, and transcript levels are increased in the *pin1* mutant (PIN1 is a transmembrane protein involved in polar auxin transport). We are currently analyzing the role of auxin in adaxial/abaxial patterning and, specifically, its contribution to the dynamic miR166 expression pattern.

Establishment of Determinacy during Organ Development

M. Guo

This work was performed with contributions from Vanessa Ringgold of the Undergraduate Research Program at CSHL.

Indeterminacy within the SAM is specified in part by the *KNOX* homeobox genes. Down-regulation of *KNOX* expression is a key factor that distinguishes stem cells and their immediate derivatives in the SAM from lateral organ founder cells. Moreover, establishment of determinacy in developing organs requires the continued silencing of the *KNOX* genes. This process involves the highly conserved MYB domain proteins ROUGH SHEATH2 (RS2) and ASYMMETRIC LEAVES1 (AS1) from maize and *Arabidopsis*, respectively. On the basis of expression and genetic analyses, we proposed that these proteins function as epigenetic regulators that, in response to a stem cell signal, keep *KNOX* genes in an "off" state during organogenesis, thus preventing differentiating cells from reverting into indeterminate stem cells. In the past few years, we have gained important new insights into the mechanism of *KNOX* gene silencing by RS2/AS1 that confirm this hypothesis. We have shown that RS2 and AS1 form highly conserved repressor complexes that include the Zn-finger transcription factor ASYMMETRIC LEAVES2 (AS2), an RNA-binding protein named RIK, and the histone chaperone HIRA.

Last year, we reported genetic data that suggested that AS1 acts as a transcriptional corepressor that may bind directly to the *KNOX* genes. We therefore used chromatin immunoprecipitation (ChIP) to determine which regions of *BP*, one of the *KNOX* genes, mediate AS1 complex binding. As a first step, we generated transgenic *Arabidopsis* lines that express an epitope-tagged version of AS1. These were used in ChIP analysis, which identified three regions within the *BP* gene that interact with the AS1 complex: two sites in the promoter and one in the coding region of *BP*. To confirm the significance of the AS1 complex-binding sites for *BP* silencing in vivo, we expressed different *BP promoter:GUS* reporter constructs in transgenic plants and analyzed expression in wild-type as well as *as1* and *as2* mutants. In our analysis, *BP* promoter fragments that included both AS1 complex-binding sites were found to contain all sequence information required for normal *BP* expression in the meristem and for AS1/AS2-mediated repression in leaves. Deletion of either AS1 complex-binding site resulted in misexpression of *BP* in wild-type leaves, verifying the importance of these sites for stable *KNOX* repression. Currently, we are using gel shift assays to analyze which proteins in the AS1 complex mediate these interactions and to more precisely define *cis* sequences required for protein binding. These data will eventually clarify the assembly of the AS1 complex in the process of stable *KNOX* gene silencing and the establishment of determinacy in developing lateral organs.

The interaction of the RNA-binding protein RIK with AS1 and RS2 complexes suggests that silencing at the *KNOX* loci may involve an RNA component. Noncoding RNAs are known to have a role in a wide range of silencing phenomena and can guide chromatin-modifying complexes to specific target loci. Using strand-specific RT-PCR, we found that an antisense transcript derived from the *BP* locus is expressed in seedlings. This antisense RNA overlaps the AS1 complex-binding site within the coding region of *BP*. Further analysis revealed that this antisense RNA is polyadenylated, and preliminary data suggest that its expression level is increased in mutants affecting the AS1 complex, including *as1*, *as2*, and *rik*. We are currently using artificial miRNA technologies to reduce antisense expression. These studies can identify the biological function of the *KNOX* antisense transcripts in recruitment of the AS1 repressor complex and the silencing of *BP*. Depending on the time and site of action of these RNAs, a first clue into the mechanism by which *KNOX* genes become down-regulated during founder cell recruitment may also be obtained.

The Role of HIRA in Plant Development

T. Phelps-Durr

This work was carried out with contributions from S. Meyerholz, Locust Valley High School.

HIRA was identified as a protein that interacts with RS2 and AS1. HIRA is a chromatin remodeling protein and was first identified in yeast as a regulator of histone gene expression.

Subsequently, it was shown that HIRA proteins modulate chromatin structure during both heterochromatic gene silencing and to control the spatial and temporal expression of specific euchromatic genes in both yeast and mammals. In *Drosophila*, HIRA is required for the decondensation of the sperm nucleus and the establishment of an epigenetic distinction between the maternal and paternal genomes. The mechanism by which HIRA establishes gene silencing is unknown. HIRA is a histone chaperone that assembles the histone variant H3.3 independently of DNA replication. In addition, HIRA can interact with histone deacetylases. Both of these activities could lead to changes in local chromatin state. We are currently testing whether *KNOX* gene silencing is associated with changes in chromatin organization or histone modifications.

In mammals, loss of *HIRA* results in gastrulation and patterning defects that lead to early embryo lethality. In addition, improper regulation of *HIRA* may contribute to DiGeorge syndrome, a cranio-facial disorder arising from improper regulation of neural crest cell derivatives during development. We are performing detailed expression analyses and are using the reverse genetic resources available in *Arabidopsis* to dissect the role of *HIRA* in plant development. In situ hybridization of *HIRA* transcripts in *Arabidopsis* vegetative meristems show that *HIRA* is expressed in all tissues, but that expression corresponds predominantly to actively dividing cells. To characterize *HIRA* expression in whole plants and throughout development, the *HIRA* promoter was fused to the GUS reporter gene and transformed into *Arabidopsis*. Characterization of several independent transgenic lines revealed that *HIRA* has a very dynamic expression pattern throughout development. Consistent with the results obtained by in situ hybridizations, *HIRA* is ubiquitously expressed in the early leaves. However, as leaves expand, *HIRA* expression becomes restricted to the indeterminate cells at the leaf margins. Expression of *HIRA* in the flowers also changes as floral development progresses. Early in floral development, expression is found at the base of flowers, in the carpels, sepals, and at the base of the anthers, whereas in older flowers, expression is limited to the developing pollen within the anthers.

Last year we reported that loss-of-function mutations in *HIRA* are embryo-lethal, but that reduced expression of *HIRA* causes formation of leaf and floral defects that resemble the defects observed in *as1* and *as2* mutants. Such *hira* mutant leaves misexpress several *KNOX* genes. Because these defects in *hira* do not result from loss of *AS1* or *AS2* expression, we proposed that AS1 and AS2 assemble into a protein complex that targets HIRA to the *KNOX* loci in organ founder cells and perhaps establishes a stable silenced chromatin state at these loci. As a result, stem cell fate remains repressed in determinate, differentiating cells of developing lateral organs. To further dissect the role of HIRA in development, we utilized the *Arabidopsis* TiLLing service to screen for EMS-induced mutations in the amino-terminal WD40-repeat protein interaction domain and the carboxy-terminal region of HIRA that interacts with RS2/AS1. Perhaps the most interesting allele is *hira-serpent*, a point mutation changing glutamate 240 to lysine in the WD40-repeat domain. Phenotypic analysis of *hira-serpent* shows that it leads to defects in pollen development and embryonic lethality. Mutant embryos abort at diverse stages during embryogenesis. The most intriguing aspect of this allele is the fact that at a low frequency, it can be recovered as a homozygous plant; surprisingly, these plants have no leaf phenotype. Detailed characterization of this allele is ongoing. It has been crossed to marker lines to determine if meristem formation, auxin localization, and root formation are perturbed. Genetic interactions between *hira-serpent* and *as1* are also under way.

Global Expression Analysis of Meristem Function and Leaf Initiation

S. Madi [in collaboration with M. Scanlon, Cornell University; P. Schnable, Iowa State University; and B. Buckner and D. Janick-Buckner, Truman State University]

Plant meristems comprise distinct histological and functional domains. For instance, the stem cells are located at the most apical tip in the so-called central zone, whereas lateral organ founder cells are located on the flanks of the SAM. Traditional genetic analyses have demonstrated the importance of this meristematic

organization for normal plant development and have led to the identification of some genes required for meristem function and lateral organ 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 LCM, which allows the isolation of transcripts from specific cell types within a 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 previously optimized 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. This past year, we further optimized the RNA extraction and amplification protocols and found that RNA collected from just a few hundred cells is sufficient for the preparation of cDNA for use in microarray analyses of gene expression.

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 intend to compare global expression patterns between approximately 20 different functional meristematic and leaf primordia domains, or experimental conditions. The following are examples of the type of comparisons we are making: (1) among the expression profiles of the stem cells, their immediate derivatives in the SAM, and the initials of lateral organs; (2) among the different tissue layers of the SAM; (3) among different stages of leaf development; (4) among the upper and lower surfaces of developing leaves; and (5) between SAMs and/or leaf primordia from developmental mutants and wild-type plants. Our initial results indicate that nearly 1300 genes are differentially expressed among the SAM, young leaf primordia, and older leaf tissues. Potential differentially expressed genes are being verified by more traditional expression analyses such as real-time RT-PCR and in situ hybridization. The function of genes of particular interest will be determined using functional genomics resources available in maize and *Arabidopsis*. Even though this project is still in its infancy, it will soon provide novel insight into meristem function and organogenesis.

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BIOINFORMATICS AND GENOMICS

Now that the full genome sequences of many organisms have been completed, scientists are increasingly turning their attention to the important task of putting this gene sequence information to work to explore biology, improve agriculture, and advance biomedical research.

This year, W. Richard McCombie and his colleagues were part of an international team that published the complete genome sequence of humanity's most important food source: rice. McCombie has also been testing the first of a new generation of DNA sequencing machines using the rice genome as a model. The goal of this project is to develop new, more rapid, and cost-effective methods for comparing DNA sequences and identifying differences between varieties of rice that might be of agricultural value. The same method would facilitate several other large-scale comparative genomic studies, including those of human genomes for the investigation of many diseases.

For plant breeders and others interested in the major crop plants—rice, corn, wheat, oats, and barley—the availability of a publicly accessible comparative genome database has the potential to speed the development of improved varieties to meet the world's increasing food demands. Lincoln Stein and his colleagues have created just such a database, dubbed Gramene. These efforts were rewarded in 2005 with a large grant to sequence and annotate the maize (corn) genome. Stein's group is also part of a public-private effort—the International HapMap Consortium—whose aim is to chart the patterns of genetic variation that are common in the world's human population. As a cover story in the journal *Nature*, this year, the researchers published the first comprehensive collection of their results called a "haplotype map" or HapMap of the human genome. The HapMap is already accelerating the search for genes involved in common diseases including cancer, heart disease, diabetes, asthma, and macular degeneration.

We tend to think of DNA as a long thread or the familiar twisted ladder, but inside cells, DNA is coated with accessories: proteins and chemical modifications that change how and when the DNA code is read. To make sense of one such modification, DNA methylation, Michael Zhang and his colleagues have identified large-scale DNA methylation patterns in the human genome and developed a new computer algorithm (Human DNA Methylation Finder) that predicts DNA methylation profiles in the human genome. This work has implications for many diseases including cancer.

The study of related protein families frequently offers clues to the function of newly discovered proteins or proteins of unknown function. Andy Neuwald uses comparative methods to study protein mechanisms through statistical analysis of the constraints imposed on protein sequences during evolution. With these methods, Neuwald and his colleagues compared a family of key enzymes called eukaryotic protein kinases (EPKs) to a set of protein kinases called "atypical protein kinases," proteins that do not appear to fit into any known protein kinase family. The result of this analysis indicates that EPK regulatory mechanisms evolved through modification of an ancestral structure. The finding provides clues to the functions of EPKs and EPK-like kinases, many of which are implicated in cancer, diabetes, and other diseases.

GENOME SEQUENCE ANALYSIS

W.R. McCombie V. Balija, J. Gergel, F. Katzenberger, M. Kramer, B. Miller, S. Muller, L. Nascimento, L. Palmer, L. Spiegel, A. Tang-Qiu, S. Teplin, M. Yu, T. Zutavern

Sequencing the Rice Genome

V. Balija, F. Katzenberger, M. Kramer, B. Miller, S. Muller, L. Nascimento, L. Spiegel [in collaboration with R. Wing, University of Arizona]

Rice is the most important crop in the world. It is the major food crop for a large part of the world's population. It is also an important model organism for the cereals, which include corn, wheat, barley, oats, and other important crops. In 2004, we finished the bulk sequencing of major segments of the rice genome as described in last year's Annual Report.

This year, we focused on two areas of the rice genome-sequencing project. We participated in the analysis and annotation of the genome. This was done as part of the Rice Annotation Project (RAP) and as part of the analysis for publication of the sequences of chromosomes 11 and 12 (Rice Chromosomes 11 and 12 Sequencing Consortia 2005) as well as the whole-genome sequencing and analysis (IRGSP 2005).

We also have been working with our collaborators at the University of Arizona to fill as many of the remaining gaps in the genome as possible. One way this is being done is by selecting clones from a closely related rice strain (*Oryza nivara*) that fill gaps in the Nipponbare target genome. These bacterial artificial chromosomes (BACs) are in turn partially sequenced, and the sequence information used to attempt to isolate the relevant Nipponbare BACs that are then sequenced. We have generated sequences from 13 Nivara BAC clones, and the Arizona group has used this sequence to probe Nipponbare fosmid and BAC libraries. Probes from 8 of the 13 Nivara clones were successful in identifying potential Nipponbare fosmid/BAC clones to help fill gaps on five different chromosomes. The Indica rice draft sequence is also currently being utilized to design probes to screen for potential BAC/fosmid clones to fill gaps in the Nipponbare sequence. In addition, we have finished 567,298 bp (CSHL + Arizona = 696,941 bp) from a set of previously unanchored Nipponbare clones, several of which either closed or extended into gaps in the genome sequence.

Also as part of the "end game" with the rice genome, we are attempting to finish clones that some of our collaborators in the International Rice Project were unable to finish. This is typically due to a high repeat content in the clones in question. We have finished 726,935 bases of such sequence and currently have seven BAC clones in progress. These final stages of the project are for the most part slow and tedious work. However, they are necessary to provide the best rice genome reference sequence possible.

Rice Transcript Analysis

V. Balija, F. Katzenberger, M. Kramer, L. Nascimento, L. Palmer, T. Zutavern

As the rice genome was nearing completion, we initiated a project to define the transcriptome of rice. As a first step in this process, we wanted to test to see if the gene structures currently annotated based on a large-scale cDNA study done by the RIKEN cDNA group (Kikuchi et al. *Science 301:* 376 [2003]), accurately and completely represented the transcripts of the genes in question.

We selected a number of genes represented in the RIKEN set and carried out RACE-PCR (rapid amplification of cDNA ends–polymerase chain reaction) on those genes using 5′-Cap-selected mRNA from rice leaves. The results are shown in Figure 1. The figure shows the somewhat surprising result that the RIKEN data set is rather incomplete in capturing the transcript initiation sites of rice genes. This is not to say that the RIKEN set is inaccurate, but rather that there seems to be a substantial number of genes having alternate initiation sites that are not represented in the database. Looking in more detail at the results, we also discovered that the genes we examined contain a substantial percentage of genes that are alternately spliced (see

FIGURE 1 Comparison of transcriptional start site in 5′-RACE products to RIKEN rice cDNAs. 5′-RACE sequences as well as RIKEN cDNAs were mapped to the rice genome. The first base of the RACE product after the RACE adapter sequence was compared to the first base of the RIKEN cDNA. A 5′-RACE product was considered similar if the first base at the 5′ end mapped to within 10 bases of the 5′ end of the RIKEN cDNA. The RACE product was considered different if it mapped further than 10 bases from the RIKEN cDNA. The overlap between these sets consists of cases where there were multiple transcripts present, some of which were similar and some of which were different from the RIKEN cDNA.

Fig. 2). These results were quite surprising given previous estimates of alternate splicing in plants of about 7% (Brett et al. *Nat. Genet. 30:* 29 [2002]; Haas et al. *Genome Biol. 3:* RESEARCH29 [2002]; Yuan et al. 2005). We are currently examining these genes in other tissues to discover the full scope of the alternate splicing, as well as analyzing other facets of our current data.

Examination of Current Gene Annotation in Human ENCODE Regions

V. Balija, M. Kramer, L. Nascimento [in collaboration with M. Zhang, Cold Spring Harbor Laboratory]

The ENCODE consortium has established 44 regions encompassing 30 Mb of the human genome (ENCODE Project Consortium *Science 306:* 636 [2004]) in which identification of all functional elements is the focus. A major facet of this project is the identification of all the genes (coding and noncoding) and promoters associated with them. Direct examination of the 5′ end of genes is valuable in verifying known exons, identifying novel exons, and recovering the full 5′ UTR (untranslated region). Additionally, experimental assessment of the 5′-end annotation will allow the transcriptional start site(s) to be accurately established, which will aid in promoter identification. With this in mind, a study was initiated with the following goals: (1) Validiate the current gene annotation in the ENCODE regions, (2) identify alternate and novel first exons, (3) identify novel splice isoforms, (4) recover the total 5′ UTR, and (5) identify alternate transcriptional start sites. All nonredundant RefSeq

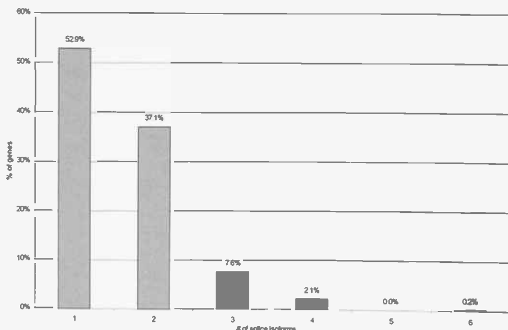

FIGURE 2 Splice variation in rice genes. The RACE products and the RIKEN cDNAs were analyzed for splice variation in terms of how many splice isoforms could be identified between the RACE and the RIKEN cDNA sequences. Alternate splice forms within the RIKEN cDNA were also included in this analysis. A total of 198 genes were identified that were represented by more than one splice isoform.

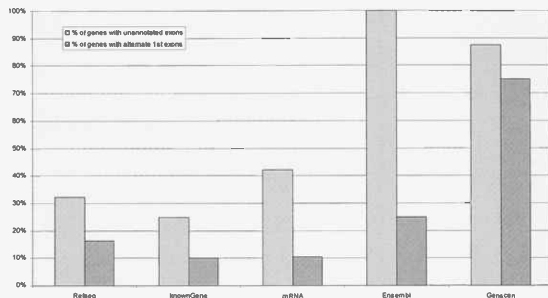

FIGURE 3 5′-end annotation of ENCODE region genes and gene predictions is incomplete. The RACE-PCR fragments from each category were aligned to the May 2004 build of the human genome using BLAT (Kent et al. *Genome Res. 12:* 996 [2002]). The percentage of genes in each category where exons observed in the RACE-PCR fragment that were not annotated in the respective gene model is shown. The number of genes where alternate first exons were observed in the RACE-PCR fragment is also depicted.

gene models (342 genes), unique knownGenes not associated with RefSeq gene models, a random sampling of mRNA sequences (37 mRNAs), and more than 200 gene predictions were chosen for amplification by 5′ RACE from 20 pooled human tissues/stages.

Analysis of the RACE products reveals that a large number unannotated and novel exons were present that were represented in the associated gene model or gene prediction model (Fig. 3). Further analysis revealed that novel alternatively spliced isoforms were also present in the RACE products (Fig. 4). These data indicate that the current state of annotations in the ENCODE regions is incomplete.

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Category	*# of genes recovered*[1]	*# of genes with multiple splice isoforms*	*# of total isoforms identifed*	*% of isoforms that are novel*
Refseq	251	84	318	22%
knownGene	20	5	22	14%
mRNA	19	7	14	43%
Ensembl	4	4	9	33%
Genscan	8	8	11	55%

FIGURE 4 Multiple splice isoforms observed in all categories. Genes in each category were analyzed to determine the level of splice variation that was observed in the RACE-PCR fragment.

PREDICTING PROTEIN MECHANISMS THROUGH BAYESIAN INFERENCE OF EVOLUTIONARY CONSTRAINTS

A.F. Neuwald N. Kannan

A major goal of structural biology is to understand protein mechanisms in atomic detail within the context of the living cell. Such an understanding is important for drug design, for protein engineering, and for generally advancing technologies relevant to agriculture, the environment, and human disease. It is also extremely valuable in advancing the emerging field of nanotechnology by revealing basic principles underlying nature's designs through reverse engineering.

Despite remarkable progress in determining protein structures, many aspects of protein mechanisms remain unclear. Although these mechanisms can only be determined experimentally, the design of such experiments is itself nontrivial and requires that one first formulate the right hypotheses. Such hypotheses are not formulated in a conceptual vacuum, however, but rather, they are based on clues obtained from preliminary observations. The evolutionary constraints imposed on protein sequences are a potential source of information in this regard inasmuch as they are due to and thus reflect these mechanisms. Moreover, because natural selection imposes these constraints on the genomic sequences of living organisms within their native environments, such information lacks the artifactual biases sometimes associated with in vitro experimental systems or with in vivo cell cultures and may reveal functionally critical features that have been overlooked due to the inherent limitations of current experimental methods. Thus, the examination of evolutionary constraints, in light of structural and other biological data, can suggest feasible hypothetical mechanisms and thereby aid experimental design.

Science works by obtaining information about what we cannot see, the hidden mechanisms underlying natural phenomena, through an analysis of what we can see, experimental "observables." We seek to obtain such information about underlying protein mechanisms through computational analysis of empirical sequence and structural and taxonomic data. To do this, we use a Bayesian approach, where ideally we would determine the probabilities associated with various hypothetical protein mechanisms given these data. Currently, this is impossible to do, however, because too little is known about the correspondence between protein mechanisms and the observables. On the other hand, evolutionary constraints, which reflect underlying mechanisms, are straightforward to model based on conserved sequence patterns. Thus, we statistically infer evolutionary constraints based strictly on empirical observations, but interpret these constraints in light of structural and biochemical data.

Although methods for analysis of sequence constraints have been around for a long time, Bayesian approaches can characterize sequence constraints in statistically rigorous and previously unexplored ways, leading to new insights. Bayesian approaches to scientific inference have three advantages: (1) Given the number of empirical observations made, they provide an explicit measure of how certain we may be about specific hypotheses, (2) they allow computers to make (statistical) discoveries by directly applying the scientific method to nonhypothesis-driven data, and (3) when provided with sufficient data, they can infer correlated properties of complex biological systems, which is very difficult to do through single-hypothesis experimentation. This year, we have continued to develop Bayesian approaches while applying them to two major classes of proteins: AAA+ ATPases and protein kinases. AAA stands for *A*TPases *a*ssociated with a variety of *a*ctivities.

Analysis of Eukaryotic DNA Clamp Loader AAA+ ATPases

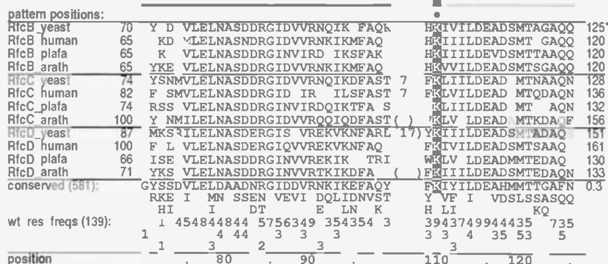
A. Neuwald

In higher organisms (the eukaryotes), five distinct replication factor C (RFC) AAA+ ATPases associate with each other to form an RFC complex involved in the loading of a circular clamp onto DNA. This clamp has an important role in keeping polymerase from falling off during DNA replication. In archaebacteria (primitive organisms from which the eukaryotes are believed to have evolved), typically, only two types of RFC ATPases, however, make up the clamp loader complex: one copy of one subunit and four copies of

the other subunit. This implies that during evolution, one of these archaeal clamp loader subunits has diverged into four distinct eukaryotic subunits. Each of the eukaryotic subunits thus has been subject to slightly different selective constraints due to their functional specialization. My analysis, which was published this year, characterized the constraints imposed on these distinct categories of clamp loader ATPases in order to predict critical aspects of the clamp loader machinery.

This analysis suggests that one of these RFC ATPases possesses a triggering component for initiating DNA-dependent ATP hydrolysis and, as a result, loading of the clamp onto DNA. It also suggests that starting with this ATPase subunit, three other RFC ATPases are sequentially activated through propagation of a series of conformational switches in which a conserved (positively charged) arginine moves into contact with (negatively charged) DNA thread through the center of the RFC/clamp complex and thereby swings away from a position in which it disruptively interacts with key catalytic residues. Notably, these conserved structural features explain the dependence of ATP hydrolysis by the clamp loader on association with DNA. Finally, this analysis revealed that by far the one feature that most distinguishes clamp loader ATPases from other AAA+ ATPases is a conserved lysine (Fig. 1, top) that electrostatically interacts with the center of the clamp-binding region of RFC subunits (Fig. 1, bottom). Moreover, this lysine is highly buried in those ATPases that are bound to the clamp in the RFC complex crystal structure, which is very unusual for a lysine that does not have a critical functional role. Thus, this lysine appears to mediate association of the clamp loader with the clamp.

AAA+ ATPases are a vast class of functionally diverse proteins involved in the assembly, operation, and disassembly of a wide variety of cellular complexes. A number of these proteins are associated with human diseases such as rhizomelic chondrodysplasia punctata, a rare autosomal recessive disorder with many associated medical complications; hereditary spastic paraplegia; the human neurologic disease



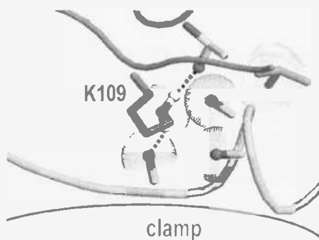


FIGURE 1 A lysine residue that most distinguishes clamp loader subunits from other AAA+ ATPases. (*Top*) Alignment quantifying (via the histogram) the constraint imposed on this lysine (K109 in the RFC B subunit). The consensus residues and integers below the alignment summarize the conserved features of 581 clamp loader subunits (a 9 below position 109, for example, indicates that lysine [K] is 90–100% conserved at this position). (*Bottom*) Structural features associated with the conserved lysine. The side chain of this lysine electrostatically interacts with the center of the region of contact between RFC and the DNA clamp.

early-onset torsin dystonia; and the neurologic disorders Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease. Hence, this analysis is of biomedical importance inasmuch as it sheds light on the general principles involved in the coupling of ATP hydrolysis by AAA+ ATPases to coordinated conformational changes linked to corresponding cellular functions.

Analysis of Eukaryotic Protein Kinases

N. Kannan, A. Neuwald

This year, we also published a comparative analysis of eukaryotic protein kinases (EPKs), distantly related EPK-like kinases, and very distantly related atypical protein kinases. EPKs have important roles in cellular signaling pathways and have been implicated in various diseases, including cancer and diabetes. A main interest in studying these proteins is to glean insights into their underlying mechanisms with a view to drug design. Indeed, this work is being conducted in close collaboration with the laboratory of Dr. Susan Taylor (Department of Chemistry, Biochemistry, and Pharmacology at the University of California at San Diego), who is now following up experimentally on our predictions.

A comparison of the evolutionary constraints imposed on these three types of kinases suggests that EPK regulatory mechanisms evolved through elaboration of an ancient structural component and provides clues regarding common regulatory themes. The most distinctive features of this component include three ancient features: a characteristic (HxD) motif adjoined to the protein kinase catalytic loop, another (DFG) motif adjoined to the activation loop, and a helix (the F-helix) located below the catalytic loop (to which it is linked via a conserved aspartate). Detailed evolutionary and structural analyses suggest that these features are associated with conformational changes and with coordinated movements associated with phosphate transfer and ADP release (the two products of ATP hydrolysis). The histidine of the HxD motif may be a focal point for signal integration inasmuch as it both associates with key catalytic elements and influences the DFG region, which can undergo dramatic conformational changes. Moreover, the EPKs (as opposed to these two other classes of kinases) have acquired additional structural features linking this ancient component both to likely substrate-interacting regions at either end of the F-helix and to three regions undergoing conformational changes upon kinase activation: the activation segment, the nucleotide-binding pocket, and another helix (the C-helix) implicated in kinase regulation. Hypothetical mechanisms suggested by this analysis have provided direction for ongoing studies on these modes of regulation.

Extensions of Our Bayesian Methods

A. Neuwald

This year, I have also initiated further extensions of our Bayesian approaches in several ways. In collaboration with statisticians Jun Liu at Harvard, Wally Gilks at Cambridge, and Kanti Mardia at Leeds, I have initiated the development of statistical models for structural analysis of evolutionary constraints. (Previously, statistical analyses were limited to sequence constraints.) I aim to infer those hypothetical structural conformations that can best explain the evolutionary constraints imposed on the corresponding protein sequences. This project requires concurrent generation of protein structural models and the use of molecular dynamics procedures to explore the "space" of hypothetical models. We are also developing statistical procedures involving multiple categories of sequence constraints (right now, analyses are limited to two categories). Although this work is still in a very early stage, very promising preliminary results have been obtained for bacterial γ clamp loader subunits.

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COMMUNITY ACCESS TO GENOME RESOURCES

L. Stein

S. Avraham, S. Cain, P. Canaran, F. Carvalho, N. Chen, B. Faga, T. Fiedler, G. Gopinathrao, T. Harris, L. Krishnan, C. Liang, C. Maher, S. McKay, S. Michaelsen, L. Ren, S. Schmidt, M. Tello-Ruiz, M. Tsang, G. Wu, C. Youens-Clark, W. Zhao

THE HUMAN HAPLOTYPE MAP

The International HapMap Project (www.hapmap.org) is an international project to map out regions of common genetic variability in the human genome by genotyping three major world populations at a resolution of one marker every 2000 bases of sequence. The resulting "haplotype map" will greatly reduce the cost of genetic association studies to find cancer susceptibility genes and other disorders with genetic components.

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 single-nucleotide polymorphisms (SNPs) to the 11 genotyping centers; coordinate data submission, quality checks, and quality control; and manage the public release of project data. The HapMap Web site, which was developed in our lab, describes the project in the five 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.

During 2005, we completed the HapMap and published the complete data set of 1 billion genotypes and their inheritance patterns on the CSHL Web site (Fig. 1). A paper describing the map and its analysis was featured on the cover of *Nature*, and our findings became the basis for roughly a dozen satellite articles describing the implications of the map. In addition to its medical research uses, the HapMap provides an insight into the evolutionary history of the human population. It confirms the "out of Africa" hypothesis and identifies multiple locations in the human genome that have been sites of adaptation by migrant populations as they adapted to different environmental conditions.

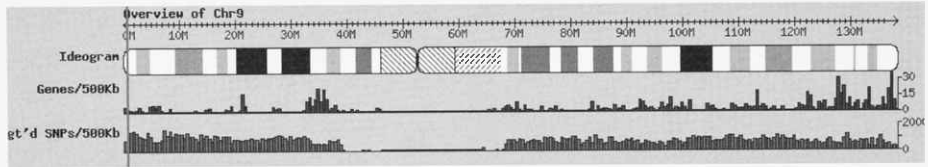
GRAMENE: A COMPARATIVE MAPPING RESOURCE FOR GRAINS

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 nonrice crop they are studying. Hence, the resource allows 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 expressed sequence tags (ESTs) to the rice genome, allowing gene predictions to be further refined based on cross-species comparisons. In previous years, we created an integrated map of the rice and maize genomes. This year, we extended that work by adding integrated maps of *Arabidopsis* and sorghum. These integrated maps allow 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.

A major achievement was the release of a "diversity module" for Gramene. This module holds information about the evolution of rice and maize by tracking patterns of genetic changes in domestic and wild populations. By characterizing these patterns, one can learn which regions of the rice and maize genomes were selected for during domestication and improvement. This information, in turn, can be used to breed new robust varieties.

We also used the information in the Gramene database to identify previously unknown microRNAs (miRNAs) in the rice and maize genomes. miRNAs are thought to be responsible for regulating key events during development and maturation. In addition to providing important information about the evolutionary history of miRNA families, this information will



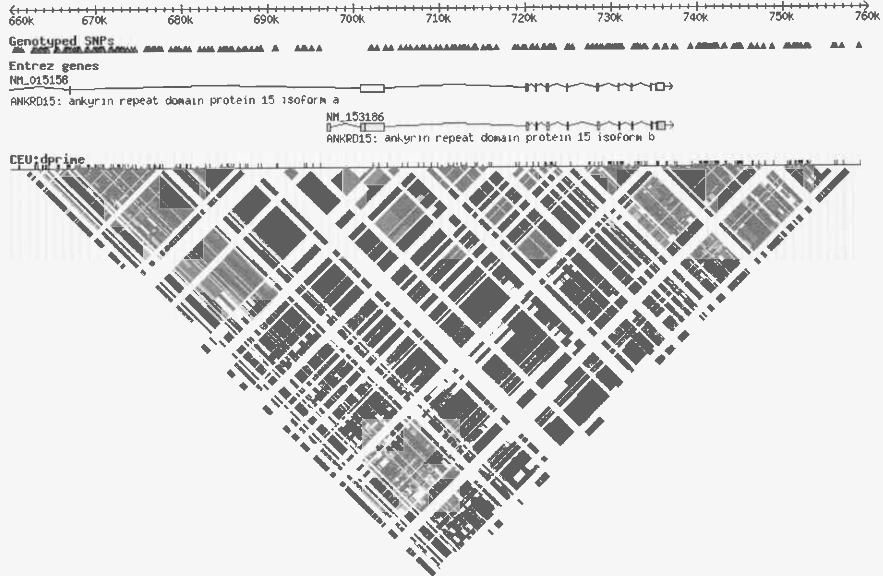


FIGURE 1 Visualizing human variation patterns with HapMap. Above is a page from the International HapMap Project Web site developed by Lincoln Stein and colleagues. The map shows an excerpt from the end of chromosome 9 (*upper panel*). The detailed view shows all of the SNPs characterized during the project, two alternative splice forms of a gene in the region, and a large triangular graph indicating patterns of coinheritance of the SNPs in the individuals genotyped during the project.

be a powerful tool for selective breeding and crop improvement.

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

Our lab continues to be a major developer and maintainer of the WormBase database (www.wormbase.org), an online 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 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.

During 2005, we have enhanced WormBase by adding mapping and sequencing information from the genome of *C. remanei*, a sister species of *C. elegans* that diverged approximately 100 million years ago. This information has allowed us to extend the set of target genes thought to be regulated by *daf-19*, a transcription factor that is key to the differentiation of sensory neurons. The significance of this finding is that many human genes that are evolutionarily related to these *daf-19*-regulated target genes are responsible for Bardet-Beidl Syndrome (BBS), a congenital form of blindness and mental retardation. Our findings greatly

increased the number of BBS candidate genes. In fact, soon after we developed our list of candidate human genes, one of them was clinically confirmed to be responsible for BBS in a group of human patients. Thus, comparative genomics in the nematode can lead to findings directly relevant to human disease.

REACTOME

Reactome (www.reactome.org) 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.

Reactome is organized like a review journal. Bench biologists are invited to create modules that summarize a particular aspect of their field. Currently summations include DNA replication, transcription, translation, intermediary metabolism, the cell cycle, RNA splicing, and hemostasis, together covering about 10% of the human division of SwissProt. Many more modules are under way. Modules are similar to minireviews, except that each paragraph of text is reduced to a series of logical assertions that are entered into a database of processes and macromolecules. The database is then used to drive a Web site that can be browsed like a textbook or searched with queries to discover pathways and connections.

During 2005, we brought the number of genes curated in Reactome to just under 1200, covering about 11% of the annotated portion of the human genome. We also added a new interface that allows users to interpret microarray data sets based on where affected genes are placed in the reaction map.

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*).

By an informal count, several hundred laboratories are now using one or more of the GMOD tools, and increasing numbers of new databases are proposing to adopt GMOD for their infrastructure.

FIGURE 2 Visualizing genome-scale patterns with GMOD. Above is a diagram generated by the GMOD Karyotype tool. This tool allows large amounts of quantitative data to be projected onto a representation of the whole genome. In this case, the diagram is displaying patterns of gene density. It demonstrates that there are regions of the genome that are relatively gene rich, and others that are relatively gene poor. The causes of this unequal distribution of genes remains a topic of investigation.

During 2005, we added new genome-wide visualization tools to GMOD, including one that allows the user to view very large quantitative data sets onto a depiction of the human chromosome set (Fig. 2).

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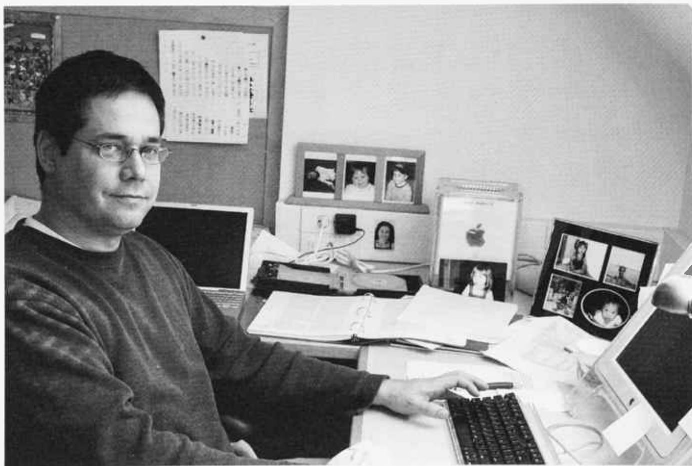

Sheldon McKay

COMPUTATIONAL GENOMICS

M.Q. Zhang	A. Agarwala	E. Santo	Z.Y. Xuan
	G.X. Chen	D. Schones	C.L. Zhang
	D. Das	A.D. Smith	F. Zhao
	N. Dimitrova	P. Sumazin	X.Y. Zhao
	S. Kamalakaran	J.H. Wang	

In the last year or so, several members have left the lab and moved on to their new careers: Nila Banerjee graduated and became a postdoctoral fellow at Columbia University near the end of 2004; Jinhua Wang became a bioinformatics research scientist in Memphis, Tennessee in the beginning of the year; Fang Zhao left in March and is now a consultant for Merck; Raj Das left in May to work for Eli Lilly; Cizhong Jiang left in June and is a research associate at the University of North Carolina; and in July, Debo Das became a research associate at the Lawrence Berkeley Laboratory and Pavel Sumazin moved on to become an assistant professor at Portland State University in Oregon.

Our main achievements last year were the (1) construction of a human, rat, and mouse promoter database that not only contains well-annotated promoters, but also predicts potentially novel promoters based on both cDNA and comparative genomic analysis; (2) further integration of motif analysis tools for finding tissue-specific transcription-factor-binding sites when combining genomic sequences with microarray data; (3) study of large-scale structure of human brain DNA methylation patterns and development of a support vector machine prediction algorithm (in collaboration with Columbia groups); (4) investigation of exonic splicing enhanced distribution (in collaboration with the Krainer lab here at CSHL) and splicing array classification of tumors (in collaboration with the University of California, San Diego and Illumina); and finally, (5) discovery of a new class of noncoding RNAs (in collaboration with the Spector lab here at CSHL).

CSHLmpd: Cold Spring Harbor Laboratory Mammalian Promoter Database

Z.Y. Xuan, F. Zhao, J.H. Wang, G.X. Chen, M.Q. Zhang

Large-scale and high-throughput genomics research needs reliable and comprehensive genome-wide promoter annotation resources. We have conducted a systematic investigation on how to improve mammalian promoter prediction by incorporating both transcript and conservation information. Using a well-characterized set of 8949 human genes with known promoters and with orthologous genes in rodents, we found that for the 5′-truncated cDNAs (1) the incorporation of transcript information can enhance specificity (Sp) and sensitivity (Sn) of de novo promoter prediction by 11% and 3%, respectively and (2) homologous genomic sequence comparison can further increase Sp and Sn by 27% and 2%, respectively. For non-CpG-related promoters in particular, the Sp can be improved from 5% to 55% with Sn only dropped 4%. This enabled us to build a better multispecies promoter annotation pipeline and hence to create CSHLmpd (Cold Spring Harbor Laboratory Mammalian Promoter Database) for the biomedical research community, which can act as a starting reference system for more refined functional annotations. (http://rulai.cshl.edu/CSHLmpd2/).

Mining ChIP-Chip Data for Transcription Factor and Cofactor-binding Sites

A.D. Smith, P. Sumazin, D. Das, M.Q. Zhang

Single motifs and motif pairs were identified that can be used to predict transcription factor localization in ChIP-chip data and gene expression in tissue-specific microarray data. We developed methodology to identify de novo individual and interacting pairs of binding-site motifs from ChIP-chip data, using an algorithm that integrates localization data directly into the motif discovery process. We combine matrix-enumeration-based motif discovery with multivariate regression to evaluate candidate motifs and identify motif interactions. When applied to hepatocyte nuclear factor (HNF) localization data in liver and pancreatic islets, our methods produce novel motifs or improved known motifs. All motif pairs identified to predict localiza-

tion are further evaluated according to how well they predict expression in liver and islets and according to how conserved the relative positions of their occurrences are. We find that interaction models of HNF1 and CDP/Cux (CCAAT-displacement protein/cut homeobox) motifs provide excellent prediction of both HNF1 localization and gene expression in liver. Our results demonstrate that ChIP-chip data can be used to identify interacting binding-site motifs.

Distribution of SR Protein Exonic Splicing Enhancer Motifs in Human Protein-coding Genes

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

Exonic splicing enhancers (ESEs) are pre-mRNA *cis*-acting elements required for splice site recognition. We previously developed a Web-based program called ESEfinder that scores any sequence for the presence of ESE motifs recognized by the human SR proteins SF2/ASF, SRp40, SRp55, and SC35 (http://rulai.cshl.edu/tools/ESE/). Using ESEfinder, we have undertaken a large-scale analysis of ESE motif distribution in human protein-coding genes. Significantly higher frequencies of ESE motifs were observed in constitutive internal protein-coding exons, compared with both their flanking intronic regions and with pseudo exons. Statistical analysis of ESE motif frequency distributions revealed a complex relationship between splice site strength and increased or decreased frequencies of particular SR protein motifs. Comparison of constitutively and alternatively spliced exons demonstrated slightly weaker splice site scores, as well as significantly fewer ESE motifs, in the alternatively spliced group. Our results underline the importance of ESE-mediated SR protein function in the process of exon definition, in the context of both constitutive splicing and regulated alternative splicing.

Profiling Alternative Spliced mRNA Isoforms for Prostate Cancer Classification

C.L. Zhang, M.Q. Zhang [in collaboration with the Fu lab at the University of California, San Diego and the Fan lab at Illumina]

Prostate cancer is one of the leading causes of cancer illness and death among men in the United States and worldwide. There is an urgent need to discover good biomarkers for early clinical diagnosis and treatment. Previously, we developed an exon-junction microarray-based assay and profiled 1532 mRNA splice isoforms from 364 potential prostate-cancer-related genes in 38 prostate tissues. Here, we investigate the advantage of using splice isoforms, which couple transcriptional and splicing regulation, for cancer classification. As many as 464 splice isoforms from more than 200 genes are differentially regulated in tumors at a false discovery rate (FDR) of 0.05. Remarkably, about 30% of genes have isoforms that are called significant but do not exhibit differential expression at the overall mRNA level. A support vector machine (SVM) classifier trained on 128 signature isoforms can correctly predict 92% of the cases, which outperforms the classifier using overall mRNA abundance by about 5%. It is also observed that classification performance can be improved using multivariate variable selection methods, which take correlation among variables into account. These results demonstrate that profiling of splice isoforms is able to provide unique and important information that cannot be detected by conventional microarrays.

Large-scale Structure of Genomic Methylation Patterns and a Human Brain DNA Methylation Prediction Algorithm

R. Das, N. Dimitrova, M.Q. Zhang [in collaboration with the Bestor and Ju labs at Columbia University]

The mammalian genome depends on patterns of methylated cytosines for normal function, but the relationship between genomic methylation patterns and the underlying sequence is unclear. We have characterized the methylation landscape of the human genome by global analysis of patterns of CpG depletion and by direct sequencing of 3073 unmethylated domains and 2565 methylated domains from human brain DNA. The genome was found to consist of short (<4 kb) unmethylated domains embedded in a matrix of long methylated domains. Unmethylated domains were enriched in promoters, CpG islands, and first exons, whereas methylated domains comprised interspersed and tandem-repeated sequences, exons other than first exons, and nonannotated single-copy sequences that are depleted in the CpG dinucleotide. The enrichment of regulatory sequences in the rela-

tively small unmethylated compartment suggests that cytosine methylation constrains the effective size of the genome through the selective exposure of regulatory sequences. This buffers regulatory networks against changes in total genome size and provides an explanation for the C value paradox, which concerns the wide variations in genome size that scale independently of gene number. This suggestion is compatible with the finding that cytosine methylation is universal among large-genome eukaryotes; many eukaryotes with genome sizes <5 x 10(8) bp do not methylate their DNA. We have also developed a computational pattern recognition method that is used, for the first time, to produce a human brain methylation map. This method can be applied both to CpG islands and to non-CpG island regions. It computes the methylation propensity for an 800-bp region centered around a CpG dinucleotide based on specific sequence features within the region. We tested several classifiers for classification performance, including K-means clustering, linear discriminant analysis, logistic regression, and support vector machine (SVM). The best performing classifier uses the SVM approach. Our program (called *HDMFinder*) presently has a prediction accuracy of 86%, as validated with CpG regions for which methylation status has been experimentally determined. Using *HDMFinder*, we have depicted the entire genomic methylation patterns for all 22 human autosomes.

Regulating Gene Expression through RNA Nuclear Retention

Z.Y. Xuan, M.Q. Zhang [in collaboration with the Spector lab, Cold Spring Harbor Laboratory]

In collaboration with the Spector lab, we have discovered a new class of noncoding RNAs (see description in the Spector lab report).

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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 4 years on projects 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), Scott Lowe (1995), and Marja Timmermans (1998) 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 Grossiklaus (1994) was a member of our faculty before leaving to join the Friedrich Miescher Institut in Basel, Switzerland in 1998. Terence Strick joined the Laboratory in 2000 after earning his Ph.D. in molecular and cellular biology at Ècole Normale Supèrieure in Paris with David Benisimon and Vincent Croquette. Terrence left to join the l'Institut Jacques Monod at the Centre National de la Recherche Scientifique.

The Laboratory currently has three CSHL Fellows, Gilbert (Lee) Henry, who joined the Laboratory in 2000, and Patrick Paddison and Ira Hall, both of whom joined the Laboratory in 2004. 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 the molecular biology of taste receptor cell differentiation. Patrick Paddison was a graduate student at the Watson School of Biological Sciences here at CSHL, where he worked in Greg Hannon's lab generating an RNA interference (RNAi) library. Patrick's research goal is to functionally map the genetic requirements for embryonic stem (ES) cell specification. Ira Hall was also a graduate student at the Watson School of Biological Sciences, where he worked in Shiv Grewel's lab on the biology of small RNAs and RNAi. He is currently interested in forming a descriptive analysis of mammalian genomic instability and plasticity, and in characterizing the contribution of DNA copy-number polymorphisms to genetic variation.

P.J. Paddison
I. Hall
L. Henry

Genes Required for Stem Cell Specification

P.J. Paddison

Mouse embryonic stem (ES) cells are cell lines derived from preimplantation embryos that can be expanded in culture while retaining the functional attributes of pluripotent early embryo cells. Mouse ES cells can be induced to differentiate in vitro within complex cellular aggregates called embryoid bodies. Previous studies have demonstrated that embryoid bodies undergo a developmental program which recapitulates many of the early events of mammalian embryogenesis with respect to the kinetics of development and differential gene expression. Fully differentiated somatic cell types, which in the developing mouse embryo are derived from precursor cells in each of the three primary germ layers (i.e., ectoderm, endoderm, and mesoderm) can now be specified in vitro from embryoid bodies.

At my bench, the initial goal of the research is to functionally map the genetic requirements for ES cell specification. To this end, I will introduce genetic lesions into ES cells that serve to block individual gene function and ask whether cell specification is also blocked at a particular stage: pregerm, germ, or postgerm layer. Presumably, blocks occurring early on during lineage commitment will affect most lineages, whereas those occurring later will only affect cells derived from particular lineages (e.g., neural-ectodermal).

To block individual gene functions in ES cells and also "stage" particular developmental lesions, I will

employ the CSHL genome-wide RNA interference (RNAi) libraries (Paddison et al. 2004) in combination with lineage-specific mouse ES cell reporter lines. Hopefully, these studies will not only offer new insight into stem cell biology and mammalian development, but also provide a toe-hold for preclinical applications of stem cells in cell replacement therapies.

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Investigation of DNA Copy-number Fluctuation Using Genomic Microarrays

I. Hall, C. Egan

We are interested in pursuing a genome-wide examination of genetic instability and plasticity and in characterizing the contribution of DNA copy-number polymorphisms (CNPs) to genetic variation. The arrangement and copy number of chromosomal segments may vary between species, strains, and individuals, and spontaneous DNA rearrangements are recognized to be causal in the clonal evolution of cancers and in the etiology of certain human diseases. Ancestral sequence relationships within and between mammalian genomes indicate a major architectural role for duplication and deletion in shaping genomes over evolutionary time, but little is known about how these processes contribute to genetic variation across more rapid time scales within normal populations and organisms. We aim to use the laboratory mouse as a model system to investigate the fundamental properties of DNA copy-number fluctuation in mammals utilizing a form of comparative genome hybridization termed representational oligonucleotide microarray analysis (ROMA) (Lucito et al., *Genome Res. 13:* 2291 [2000]).

Internal relationships within sequenced genomes show that a significant portion (2-5%) of chromosomal DNA in mammals is contained within segmental duplications (defined as stretches of DNA within a single genome >1 kb in length and >90% identical to one another), and duplicative events underlie the historical amplification and diversification of many important gene families. Sequence comparisons are essential to identify ancient copy-number changes, but they are not well suited to detect recent and ongoing genetic events because a complete genome sequence (1) is usually derived from a single individual, (2) relies upon assembly methods that are confounded by large identical repeats, and (3) is still impractical to attain for large genomes. Traditional molecular techniques are inevitably limited (and directed) to a small number of loci, and cytogenetic methods lack sufficient resolution to detect most changes. ROMA allows for the simultaneous detection of CNP and restriction-fragment-length polymorphisms (RFLPs) between any two related DNA samples through comparative hybridization of simplified genomic representations to high-density oligonucleotide microarrays. This technique assays a large portion of the genome in a relatively unbiased manner, and it requires only that a complete genome sequence exists for the organism in question. A number of recent studies have cataloged extensive copy-number differences between humans, indicating that CNPs may account for a substantial portion of existing genetic variation.

The apparent evolutionary importance and contemporary prevalence of segmental copy-number variation raises fundamental questions. How often do new CNPs arise? Do CNPs arise through a random process? Does copy number generally reflect the ancestry of a locus? What are the prevalent mechanisms of duplication and deletion? Are different chromosomal regions or classes of DNA sequences more variable than others? Do distinct cells or cell types of an organism contain the same genetic material?

The laboratory mouse is an ideal model system for such investigations in that the human and mouse genomes appear to have been shaped by similar mutational forces, and the mouse offers the significant technical advantages of controlled crosses, experimental manipulation, and a known breeding history.

GENETIC DIVERSITY IN INBRED MICE

During the past century, a large number of phenotypically diverse inbred strains have been derived from a small number of founder mice through brother-sister mating. Because these founders were mixed descendants of Asian and European subspecies, each modern inbred line contains a unique, recombinant mixture of chromosomal segments of distinct genetic origin. These genetic differences are thought to underlie the many interesting physical and behavioral traits for which modern strains vary.

To gain an understanding of the current genetic composition of the laboratory mouse and to evaluate the utility of ROMA as a tool for mouse genetics, we have profiled the genomes of a number of commonly used inbred strains. Using a microarray containing approximately 83,000 probes, we have identified about 15,000 ROMA polymorphisms from 12 strains. More than one third of these markers can be observed in a single strain comparison, and further investigation with independent methods indicates that about one fifth of them are CNPs (the remainder are RFLPs). These markers are distributed throughout the genome in a punctate, highly nonrandom fashion, and in collaboration with S. Sridhar (Carnegie Mellon), we have developed a segmentation algorithm to identify ancestrally divergent haplotypes and CNPs within our data (this model is based on previous work by L. Muthuswamy and M. Wigler here at CSHL). We have compared ROMA polymorphisms to a curated set of 3.8 million single-nucleotide polymorphisms (SNPs) and found that while the majority of ROMA differences are ancient in origin and mirror the distribution of SNPs, significant genomic changes have occurred since the establishment of inbred lines in the early part of the 20th century.

GENOME STABILITY AND PLASTICITY

To assess the importance of segmental duplication and deletion in generating de novo genetic variation, we are examining genome stability through normal cycles of somatic development and germ-line transmission by measuring the frequency and pattern of DNA copy-number alterations in diverse cellular lineages of the mouse.

Mutation in the germ line is the basis for the introduction of new alleles to a species and is relevant to our understanding of evolution, population structure, and sporadic human disease. Previous estimates of the germ-line mutation rate have relied on the observation of spontaneous loss of function at a small number of genes and do not adequately distinguish between classes of mutations. The rates of de novo segmental duplication and deletion are not known. Our analysis of genetic diversity described above utilized multiple individuals from each strain and thus represents a test of the purportedly homozygous and uniform nature of the inbred mouse genome. We found that inbred mice are extremely inbred, but rarely identical. We did not observe residual heterozygosity, but in the majority of cases, we encountered one or more spontaneous CNPs between different individuals of the same strain. A further sampling of eight C57BL/6 individuals revealed five CNPs, and a comparison of two substrains demonstrated that even very closely related lineages may differ by a large number of CNPs.

On the basis of these results, we have initiated a study to measure the rate of spontaneous copy-number change. We have collected pedigreed mice from many different substrains that have been separated from each other for 10–100 generations of inbreeding. Our preliminary results from this study indicate that genetic drift is rapid and prevalent and that CNPs arise at a surprisingly high rate compared to a single nucleotide mutation. The spontaneous genomic changes identified thus far vary dramatically in size (~1 kb to 15 mb), can encompass well-known genes and highly conserved elements, and have in notable cases independently arisen within distinct inbreeding lineages and/or recurred within a single lineage.

A description of the genetic variation normally present in somatic cells is important for a few major reasons. Each cancer is thought to begin with a single cell that has genetically diverged from its relatives, and various theories contend that the accumulation of mutations is a primary cause of aging. DNA rearrangement is also an established regulatory mechanism; our own bodies generate a staggering diversity of immunological molecules precisely through such "mutagenic" processes, and more simple genetic systems (such as various yeasts and bacteria) utilize reversible DNA rearrangements to make heritable transcriptional decisions. It is not known how common such mechanisms are in mammals. In collaboration with J. Hicks here at CSHL, we are pursuing a survey of somatic cell diversity. As a first pass, we are simply analyzing different tissues from the same individual, but we plan to obtain more pure cell populations via flow cytometry and microdissection. We are also collaborating with K. Eggan (Harvard University) to test the genetic equivalence of different cells and cell types using somatic cell nuclear transfer. We hope that these

studies result in an estimate of clonal variegation in mammals and help to identify unstable or potentially hypervariable regions of the genome.

Structural and Functional Studies of the Vertebrate Taste Bud

L. Henry, M. Siddiqui

We are interested in the molecular biology of taste receptor cell differentiation. The mammalian taste bud is a dynamic structure consisting of mitotically active progenitor cells at its periphery and differentiated taste receptor cells within its core. The basic goal of our research is to understand how the daughters of the progenitors select a particular fate. As different receptor cells are known to respond to different classes of tastants, we hope that understanding this differentiation process will help to illuminate both details of taste bud structure and function. Toward this end, we have undertaken an exhaustive effort to define the cellular diversity of the murine taste bud at the level of transcription. To do this, single-cell profiling is being used to analyze the transcriptomes of taste receptor and progenitor cells. We are currently developing a novel procedure to produce single-cell profiles that rely on the attachment of single-cell levels (picogram) of mRNA to magnetic beads. The immobilization of picogram amounts of nucleic acid allows manipulations, free from complicating purification procedures, to be performed that would usually require microgram amounts of material. Various aspects of this procedure have been optimized during the past year, including the identification of a novel first-strand cDNA-priming scheme that solves a key background problem associated with the procedure. The design and synthesis of a novel cleavable linker both provide flexibility for the priming oligonucleotide and a means to detach cDNA from the magnetic bead after a series of modification steps.

SOLID-PHASE mRNA AMPLIFICATION

In the past year, we have completed all of the synthetic chemistry steps required to assemble the solid-phase system used in our amplification procedure. A highly efficient five-step synthesis was developed for the production of polyethylene-glycol-based linkers of varying lengths (4–37 atoms). The linker is an ortho-nitro benzene sulfenyl (oNBS) monoprotected diamine that contains a periodate cleavable *cis*-diol proximal to the oNBS end of the molecule. We have tested a series of linkers and determined that 25 atoms is the minimal length of the molecule needed for our assay.

Thiol-modified oligomers were attached to 3-μm magnetic beads via the aforementioned linker in a four-step procedure. The protected linker was attached to the bead via a carbodiimide-based acylation. After oNBS deprotection, the distal end of the linker was adapted with a commercially available heterobifunctional linker that places a maleimide on the bead that then accepts the thiol-modified oligomer. Using this procedure, we can produce beads that bind at a minimum 10 pmoles of poly(dA).

We are currently using this bead system to amplify picogram amounts of starting material. We will test the fidelity of the system by assessing the ability of our procedure to maintain relative transcript levels in amplified versus nonamplified tester pools of mRNA.

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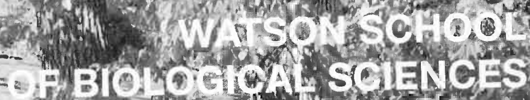
WATSON SCHOOL OF BIOLOGICAL SCIENCES



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WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

It was once more an eventful year for the Watson School of Biological Sciences! A Watson School student, Zachary Lippman, was the recipient of the Harold Weintraub Award, the Arnold and Mabel Beckman Foundation renewed—and increased—its support of our graduate program, the National Science Foundation renewed its support of our summer undergraduate program, and six more students were awarded their doctoral degrees!

The Second Watson School Graduation

April 17th saw the Watson School's second graduation ceremony. Michelle Cilia, Ahmet Denli, Elena Ezhkova, Zachary Lippman, Masafumi Muratani, and Ji-Joon Song made up the graduating class of 2005. The graduation itself was a wonderful occasion at which honorary degrees were bestowed on Drs. Daniel Koshland and Bruce Alberts, with Dr. Alberts giving the keynote address. We were further honored that the parents of Elena Ezhkova and Masafumi Muratani traveled from Russia and Japan, respectively, to attend the graduation ceremony.

The graduating class is a truly impressive group. All six have taken up postdoctoral research positions, and we wish them every success on the next leg of their scientific journey. We are also delighted to report that Zachary Lippman has been awarded a Human Frontiers Long Term Fellowship for his

(*Left to right*) Elena Ezhkova, Zachary Lippman, Michelle Cilia, Ji-Joon Song, Masafumi Muratani, Ahmet Denli

postdoctoral studies, and Ji-Joon Song was awarded a Helen Hay Whitney Fellowship for his postdoctoral studies.

Student	Thesis advisor	Current Postdoctoral Position
Michelle Cilia	David Jackson	With Dr. Sondra Lazarowitz, Cornell University
Ahmet Denli	Gregory Hannon	With Dr. Fred Gage, The Salk Institute
Elena Ezhkova	William Tansey	With Dr. Elaine Fuchs, The Rockefeller University
Zachary Lippman	Robert Martienssen	With Dr. Dani Zamir, Hebrew University of Jerusalem
Masafumi Muratani	William Tansey	With Dr. Richard Treisman, Cancer Research, United Kingdom
Ji-Joon Song	Leemor Joshua-Tor	With Dr. Robert Kingston, Massachusetts General Hospital

In addition to the six students who graduated this year, we saw another two students move on. Izabela Sujka of the entering class of 2003 decided to leave the program, after completing the requirements for a Master's degree, to move to a job in industry. Before joining our graduate program, Izabela had worked as a research technician for 2 years. She is now working as a research assistant at OSI Pharmaceuticals, in the area of cancer biology. Molly Perkins of the entering class of 2004, and her husband, relocated to Oxford, England. We are happy to report that Molly was able to transfer to a Ph.D. program at Oxford University where she will continue with her Ph.D. research under the mentorship of Dr. Andrew McMichael, working in the field of immunology.

THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 1999

Ahmet E. Denli, March 29, 2005

Processing of pri-microRNAs by the Microprocessor complex.

Thesis Examining Committee

Chair:	**Nouria Hernandez**
Research Mentor:	**Gregory Hannon**
Academic Mentor:	**Adrian R. Krainer**
Committee Member:	**Scott Lowe**
Committee Member:	**Robert Martienssen**
External Examiner:	**Gail Mandel,** Stony Brook University

ENTERING CLASS OF 2000

Santanu Chakraborty, December 9, 2005

Analysis and modeling of neural connectivity in a short-term memory system.

Thesis Examining Committee

Chair:	**Zachary Mainen**
Research Mentor:	**Carlos D. Brody**
Academic Mentor:	**Michael Wigler**
Committee Member:	**Karel Svoboda**
Committee Member:	**Bhubaneswar Mishra**
External Examiner:	**David W. Tank,** Princeton University

Elena Ezhkova, March 25, 2005

Role of the proteasome in gene control.

Thesis Examining Committee

Chair:	**Nouria Hernandez**
Research Mentor:	**William Tansey**
Academic Mentor:	**Jan A. Witkowski**
Committee Member:	**Bruce Stillman**
Committee Member:	**Rui-Ming Xu**
External Examiner:	**Jerry Workman,** Stowers Institute

Masafumi Muratani, March 31, 2005

Regulation of gene expression by the ubiquitin-proteasome system.

Thesis Examining Committee

Chair:	**David L. Spector**
Research Mentor:	**William Tansey**
Academic Mentor:	**Nouria Hernandez**
Committee Member:	**Tatsuya Hirano**
Committee Member:	**Mark Ptashne**
External Examiner:	**Michael Tyers,** University of Toronto

ENTERING CLASS OF 2001

Ji-Joon Song, April 1, 2004

Structural and biochemical studies of argonaute reveal the slicing mechanism of RISC-RNAi effector complex.

Thesis Examining Committee

Chair:	**Gregory Hannon**
Research Mentor:	**Leemor Joshua-Tor**
Academic Mentor:	**Scott Lowe**
Committee Member:	**Robert Martienssen**
Committee Member:	**Christopher D. Lima**
External Examiner:	**Craig Mello,** U. Mass Medical School

Zachary Lippman Becomes the Second Weintraub Awardee for the Watson School

Zachary Lippman

I am delighted to report that a second Watson School student, Zachary Lippman of the entering class of 2000, was a 2005 recipient of the prestigious Weintraub Award. The Harold M. Weintraub Award for excellence in graduate studies is given annually to 15 students by the Fred Hutchinson Cancer Research Center. This is the second time a Watson School student has received this international award—Amy Caudy of the first entering class was a recipient in 2003.

Alumni Spotlight

Amy Caudy

Amy A. Caudy, Ph.D., received a B.A. from Washington University in St. Louis and was a member of the first entering class of the Watson School of Biological Sciences as well as the School's first graduate. Dr. Caudy was awarded the prestigious Fred Hutchinson Cancer Research Center's Harold M. Weintraub Award and was also the recipient of a Howard Hughes Medical Predoctoral Institute Fellowship during her time at the Watson School. She completed her postdoctoral research with Dr. John Atkinson at Washington University in St. Louis and is currently a Lewis-Sigler Fellow at the Lewis-Sigler Institute for Genomics at Princeton University. Says Amy of her time at Cold Spring Harbor Laboratory:

> *CSHL is one of the most perfect places to do science that I could imagine. First, there are terrific interactions between people in diverse disciplines. The institution is just the right size to make it possible for a person working on RNAi in* Drosophila *and mammals to continually rub shoulders with neurobiologists, plant biologists, and cancer biologists. Second, CSHL makes it easy to do ambitious experiments by providing many excellent shared resource facilities. The Watson School fosters an environment of high expectations. The intellectual rigor of the* Scientific Reasoning and Logic *core course sets the stage for a research environment where graduate students can eagerly debate the latest results and plot the course for exciting new experiments with research advisors, postdocs, and other graduate students.*

The Fall Term Curriculum

Our faculty members continue to do an outstanding job developing and delivering the curriculum. As ever, the Curriculum Development and Integration Committee (CDIC), headed by William Tansey, continues to carefully monitor and develop the curriculum. This year, Josh Huang joined the CDIC, which now comprises William Tansey (Chair), Lilian Gann, Z. Josh Huang, David L. Spector, and Nicholas Tonks. For Fall 2005, the Scientific Reasoning and Logic core course has undergone some instructor turnover, although the content of many of the modules remains the same. Carlos D. Brody, Hollis Cline, and Arne Stenlund stepped down as full instructors. Z. Josh Huang, W. Richard McCombie, and Scott Powers were recruited to fill these spots. In addition, Gregory Hannon stepped down as lead instructor, but remains a course instructor, and Senthil K. Muthuswamy is now lead instructor.

For 2005, the Scientific Exposition and Ethics (SEE) core course has essentially remained the same, following on from its highly successful revamp in 2004. This course continues to attract outstanding guest instructors who this year included:

Robert Charrow	Greenberg Traurig, LLP, Washington, D.C.
Colin Goddard	OSI Pharmaceuticals, Melville, New York
Peter Petre	Fortune, New York
Philip Reilly	Interleukin Genetics, Inc., Waltham, Massachusetts
Bodo Stern	Cell Press, Cambridge, Massachusetts
David Vaux	Walter and Eliza Hall Institute, Victoria, Australia

The CDIC continues to interact closely with potential Specialized Disciplines course instructors not only to identify new topics, but to help them develop courses and their methods of assessment and to monitor workloads. For Fall 2005, the Molecules to Networks course was replaced by a neuroscience course Neuroscience: Systems and Behavior, taught by Carlos D. Brody (lead instructor) and Zachary Mainen.

ENTERING CLASS OF 2005

Patrick M. Finigan, University of California, Davis
George A. and Marjorie H. Anderson Fellow

Amy Y. Leung, Cornell University
Beckman Graduate Student

Sarahjane Locke, Wells College
George A. and Marjorie H. Anderson Fellow

Hiroshi Makino, University of St. Andrews
Elizabeth Sloan Livingston Fellow

Katherine McJunkin, Princeton University
Robert and Theresa Lindsay Fellow, Leeds Family Scholar

Nicholas E. Navin, Skidmore College
Goldberg-Lindsay Fellow

Frederick D. Rollins, Cornell University
Cashin Fellow

Oliver Tam, University of Sydney, Australia
Bristol-Myers Squibb Fellow

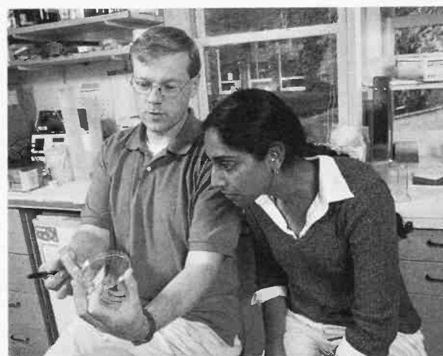
Jeremy E. Wilusz, Johns Hopkins University
Beckman Graduate Student

Entering class of 2005. (*Left to right*) Nicholas Navin, Katherine McJunkin, Amy Leung, Frederick Rollins, Hiroshi Makino, Oliver Tam, Jeremy Wilusz, Sarahjane Locke, Patrick Finigan

Entering Class of 2005

On August 29, 2005, the Watson School opened its doors for the seventh time to welcome yet another new class. Nine students—Patrick Finigan, Amy Leung, Sarahjane Locke, Hiroshi Makino, Katherine McJunkin, Nicholas Navin, Frederick Rollins, Oliver Tam, and Jeremy Wilusz—make up the Entering Class of 2005. Amy Leung was a participant in our summer undergraduate research program in 2004, and Nicholas Navin came to us having spent 2 years as a scientific programmer with Michael Wigler.

Recruiting Efforts



William Tansey and Watson School student Shraddha Pai

Recruitment of the graduate program's entering class of 2006 and of participants for our summer 2006 undergraduate research program (URP) was once more managed by Ms. Dawn Meehan, the School's admissions, recruitment, and student affairs manager. Throughout 2005, Dawn, traveled the length and breadth of the country to recruit students to our graduate and undergraduate programs. She has done an outstanding job representing Cold Spring Harbor Laboratory and the Watson School, and I am deeply indebted to her for her efforts. The table below details the different recruitment fairs and conferences the School has participated in, together with the names of faculty, students, and administrators who represented the School on these occasions.

The School is also deeply indebted to its graduate program's Admissions Committee, who review, interview, and select candidates for our doctoral program. The Admissions Committee for the 2005 entering class comprised Gregory Hannon (Chair), Carlos D. Brody, Josh Dubnau, Adrian R. Krainer, Robert Lucito, Alea A. Mills, Michael P. Myers, Karel Svoboda, Linda Van Aelst, and William Tansey (ex officio). They are a truly remarkable team!

2005 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date	WSBS Attendees/Titles
Ronald E. McNair Scholars Conference	University of Maryland, College Park	March 17–20	Dawn Meehan (Admissions, Recruitment, and Student Affairs Manager)
Wellesley College Summer Undergraduate Research Program	Wellesley College	July 20	Dawn Meehan, Dr. William Tansey (Director of Graduate Studies)
Stony Brook University Summer Undergraduate Research Program	Cold Spring Harbor Laboratory	July 22	Dawn Meehan, Dr. Lilian Clark Gann (Dean), Dr. William Tansey
University of Maryland, Baltimore County Biology Department Seminar and Meyerhoff Scholarship Program Visit	University of Maryland, Baltimore County	Sept. 14–15	Dawn Meehan, Dr. William Tansey
XVI Undergraduate Research Symposium NSF/UMET Model Institutions for Excellence	Universidad Metropolitana, San Juan, Puerto Rico	Sept. 16–17	Dawn Meehan, Dr. William Tansey
Washington, D.C. Area Universities Graduate and Professional School Fair	George Washington University	Sept. 26	Dawn Meehan
Cornell University Graduate and Professional School Day	Cornell University	Sept. 27–28	Dawn Meehan, Dr. William Tansey
New York University Graduate School Fair	New York University	Sept. 28	Dr. Alyson Kass-Eisler (Curriculum Administrator and Postdoc Program Officer), Dr. Robert Lucito (Assistant Professor)
Society for Advancement of Chicanos and Native Americans in Science (SACNAS)	Denver, Colorado	Sept. 29–Oct. 1	Dawn Meehan, Dr. William Tansey, Oliver Fregoso (Graduate Student)
Howard University Graduate School Fair	Howard University	Oct. 4	Dawn Meehan
California Institute of Technology Career Fair	California Institute of Technology	Oct. 12	Dawn Meehan, David Simpson (Graduate Student)
University of California, Berkeley Graduate School Fair	University of California, Berkeley	Oct. 13	Dawn Meehan, David Simpson
Brandeis University Graduate School Fair	Brandeis University	Oct. 19	Dr. Alyson Kass-Eisler, Dr. Tim Tully (Professor)
University of California, Davis Graduate School Information Day	University of California, Davis	Oct. 28	Dawn Meehan, Dr. William Tansey
California Forum for Diversity in Graduate Education	California State University, Sacramento	Oct. 29	Dawn Meehan, Dr. William Tansey
Hunter College Graduate School Fair	Hunter College	Nov. 1	Dr. Josh Dubnau (Assistant Professor), Shraddha Pai (Graduate Student)
Annual Biomedical Research Conference for Minority Students (ABRCMS)	Atlanta, Georgia	Nov. 2–5	Dawn Meehan, Dr. Lilian Clark Gann, Keisha John (Graduate Student), Eva Radeck (Administrative Assistant)

Academic Mentoring

The Watson School takes great pride in the level of mentoring it offers its students. One of the very special aspects in this regard is its 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 watchful guardian to look over and encourage students through the sometimes-trying process of a doctoral education. This program continues to receive much support

DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
ENTERING CLASS OF 1999			
Ahmet Denli *David Koch Fellow* **Thesis Defense: March 2005**	Adrian Krainer	Gregory Hannon	Processing of pri-microRNAs by the micro processor complex.
ENTERING CLASS OF 2000			
Santanu Chakraborty *George A. and Marjorie H. Anderson Fellow* **Thesis Defense: December 2005**	Michael Wigler	Carlos D. Brody	Analysis and modeling of neural connectivity in a short-term memory system.
Elena S. Ezhkova *Engelhorn Scholar* **Thesis Defense: March 2005**	Jan A. Witkowski	William Tansey	Role of the proteasome in gene control.
Rebecca C. Ewald *Engelhorn Scholar*	Bruce Stillman	Hollis Cline	NMDA receptor subtypes and dendritic arbor morphology.
Marco Mangone *Charles A. Dana Foundation Fellow*	Linda Van Aelst	Winship Herr	Analysis of the HCF-1 basic region and its role in sustaining cell proliferation.
Masafumi Muratani *George A. and Marjorie H. Anderson Fellow* **Thesis Defense: March 2004**	Nouria Hernandez	William Tansey	Regulation of gene expression by the ubiquitin-proteosome system.
ENTERING CLASS OF 2001			
Catherine Y. Cormier *Beckman Graduate Student* *NSF Graduate Research Fellow*	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.
Tomás Hromádka *Engelhorn Scholar*	William Tansey	Anthony Zador	Stimulus optimization in the auditory cortex.
Charles D. Kopec *Goldberg-Lindsay Fellow* *NRSA Graduate Research Fellow*	Anthony Zador	Roberto Malinow	AMPA receptor trafficking and their effect on spine enlargement during long-term potentiation.
Ji-Joon Song *Bristol-Myers Squibb Fellow* **Thesis Defense: March 2005**	Scott Lowe	Leemor Joshua-Tor	Structural and biochemical studies of Argonaute reveal the slicing mechanism of RISC-RNAi effector complex.
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, Hollis Cline	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.

from the faculty who volunteer to be academic mentors, and it has rightfully become a vital ingredient in the Watson School's success. The following are this year's new academic mentors for the entering class of 2005: Senthil K. Muthuswamy (Patrick M. Finigan); Robert Lucito (Nicholas E. Navin); William Tansey (Amy Y. Leung); Jan A. Witkowski (Frederick D. Rollins); Josh Dubnau (Sarahjane Locke); David Jackson (Oliver Tam); Hollis Cline (Hiroshi Makino); John R. Inglis (Jeremy E. Wilusz); and Terri Grodzicker (Katherine McJunkin)

DOCTORAL THESIS RESEARCH (continued)

Student	Mentor	Academic Mentor	Research Thesis Research
Beth L. Chen *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	Role of insulin receptor in the development of neuronal structure and function.
Jonathan Kui *Alfred Hershey Fellow*	David Jackson	Tim Tully	Identification and characterization of candidate memory genes in Arleekin, a *Drosophila* memory mutant.
Elizabeth Murchison *Engelhorn Scholar*	John Inglis	Gregory Hannon	Role of Dicer in mammalian development.
ENTERING CLASS OF 2003			
Hiroki Asari *Farish-Gerry Fellow*	Z. Josh Huang	Anthony Zador	Sparse overcomplete representation as a principle for computation in the brain.
Rebecca Bish *David H. Koch Fellow*	Linda Van Aelst	Michael Myers	A proteomics approach to the study of ubiquitylation.
François Bolduc *William R. Miller Fellow*	Hollis Cline	Tim Tully	Role of dFMR1 and the RNAi pathway in *Drosophila* learning and memory.
Monica Dus *Engelhorn Scholar*	John Inglis	Gregory Hannon	Characterization of the biological roles of the PIWI subfamily.
Angélique Girard *Florence Gould Fellow*	Jan Witkowski	Gregory Hannon	Role of the PIWI family and PIWI-associated small RNAs in mammalian spermatogenesis.
Christopher Harvey *David and Fanny Luke Fellow*	Adrian Krainer	Karel Svoboda	Visualization of MAPK activity in neurons.
Izabela Sujka *Beckman Graduate Student*	Marja Timmermans	Senthil K. Muthuswamy	Involvement of polarity genes in ErbB1- and ErbB2-induced tumorigenesis in vivo.
Wei Wei *George A. and Marjorie H. Anderson Fellow*	Jan Witkowski	Roberto Malinow	Activity-dependent modulation of APP processing and $\alpha\beta$ production in rat hippocampal neurons.
ENTERING CLASS OF 2004			
Daniel Chitwood *George A. and Marjorie H. Anderson Fellow*	Alea A. Mills	Leemor Joshua-Tor	Structural and biochemical characterization of the *Schizosaccharomyces pombe* RITS complex.
Galen Collins *Beckman Graduate Student*	Marja Timmermans	William Tansey	Role of ubiquitin ligases and activator destruction in transcription.
Oliver Fregoso *Seraph Foundation Fellow* *William Randolph Hearst Scholar*	Adrian R. Krainer	Michael P. Myers	Identifying the mechanism of adeno-associated virus (AAV) integration and gene targeting and development of a novel molecular biology tool.
Keisha John *Ford Foundation Fellow* *William Randolph Hearst Scholar*	Josh Dubnau	Linda Van Aelst	Identification of the molecular determinants contributing to DOCK7's role in neuronal polarity.
Shraddha Pai *Charles A. Dana Fellow*	Anthony Zador	Carlos D. Brody	Determining the neuroanatomical loci and electrical correlates of duration discrimination in the rat.
David Simpson *Beckman Graduate Student*	Scott Lowe	William Tansey	Revealing insights into cancer biology with tumor-derived mutations in c-Myc.

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS		
Student	**CSHL Research Mentor**	**Program**
Abishek Chakraborty	William Tansey	Stony Brook, Molecular and Cellular Biology
Cindy Chang	Scott Lowe	Stony Brook, Genetics
Hsueh-Cheng Chiang	Yi Zhong	Stony Brook, Neuroscience
Xingyue He	Gregory Hannon	Stony Brook, Genetics
Ingrid Ibarra	Gregory Hannon	Stony Brook, Genetics
Danny Khalil	William Tansey	Stony Brook, Genetics
Anna Kloc	Robert Martienssen	Stony Brook, Genetics
Cem Kuscu	Scott Powers	Stony Brook, Molecular and Cellular Biology
Jane Lee-Osbourne	Hollis Cline	Stony Brook, Neuroscience
Xiaofei Liu	Arne Stenlund	Stony Brook, Molecular and Cellular Biology
Prem Premsrirut	Scott Lowe	Stony Brook, Genetics
Smita Srivastava	Jacek Skowronski	National Institute of Immunology (New Delhi)
Shuying Sun	Adrian R. Krainer	Stony Brook, Molecular and Cellular Biology
Hongjae Sunwoo	David L. Spector	Stony Brook, Molecular and Cellular Biology
Xiaoyun Wu	Z. Josh Huang	Stony Brook, Genetics
Wen Xue	Scott Lowe	Stony Brook, Molecular and Cellular Biology
Yu-Ting Yang	Linda Van Aelst	Stony Brook, Molecular and Cellular Biology

Interinstitutional Academic Interactions

It is important to remember that many of the graduate students studying at Cold Spring Harbor Laboratory are not Watson School students. Indeed, the largest percentage of students are from Stony Brook University, via the programs we have shared with them for some 30 years. The Watson School provides an on-site "home" for all of these students to help them feel that they are part of the Laboratory community and to help them as much as possible with the complexities of performing doctoral research away from the parent institution. This year, the students listed above joined us.

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 that it has a diverse multi-institutional graduate student community, especially the mixing of Stony Brook University and Watson School students. The graduate student seminars, which are held each September to May, enable 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 moderated by a graduate student chair. In attendance are the graduate students and two members of a core set of six faculty mentors whose attendance rotates each week. The seminar series, which is open to the entire Laboratory community, serves three important roles. First, it gives students an opportunity to hone their oral presentation skills. Second, it provides students with an opportunity to defend their research without the assistance 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. In addition, members of the audience complete (anonymously) seminar evaluation forms that are given to the presenting students. Josh Dubnau, Terri Grodzicker, Alea A. Mills, Andrew Neuwald, Arne Stenlund, and Yi Zhong served as faculty mentors and brought a breadth of knowledge and experience to the seminar series.

Faculty Changes

One new research faculty member joined the Watson School this year. Jonathan Sebat joined us as an assistant professor. Jonathan came to the Watson School after completing his postdoctoral studies in Michael Wigler's laboratory here at Cold Spring Harbor Laboratory. Jonathan's research will focus on the role of gene copy-number variation in neurological disease. We are excited to have him here and look forward to his participation in Watson School activities.

This year we also saw the departure of three faculty members. Winship Herr, the founding dean of the Watson School, moved his laboratory to Switzerland. Winship joined the Université de Lausanne as a Professor in the Center for Integrative Genomics. His current interests focus on understanding how HCF-1 regulates cell proliferation, especially as it pertains to cancer and cell differentiation.

Nouria Hernandez, who had been an integral part of the Watson School since its inception, also moved to the Université de Lausanne this year. Nouria served on the School's Admissions Committee for 5 years and as its chair for 3 years. She was a guest lecturer in the Scientific Reasoning and Logic core course, the Mechanisms of Transcriptional Regulation: From *E. coli* to Elephants specialized disciplines course, and the Scientific Exposition and Ethics core course. Nouria served as a laboratory rotation advisor, an academic mentor, and a thesis committee chair. Nouria has joined the Université de Lausanne as a Professor and as the Director of the Center for Integrative Genomics. Her research focuses on understanding fundamental mechanisms of transcription and transcriptional regulation.

Masaaki Hamaguchi, who served as a laboratory rotation advisor, is now an Associate Professor in the Department of Biological Sciences at Fordham University. His research focuses on studying the genes responsible for cancer development.

We are deeply indebted to all three for their efforts and wish them well in their new "homes."

Graduate Students and Postdoctoral Fellow Departures

With each year comes not only new arrivals, but also departures. The following graduate students and postdoctoral fellows departed from the Laboratory during 2005:

Graduate Students

Bidisha Chattopadhyaya
Stephen D. Clow
Elena Ezhkova
Agnes Fischer
Hilde Grassmo-Wendler
Kendall Jensen
Lukasz C. Kozaczkiewicz
Andrea Leibfried
Zachary B. Lippman
Masafumi Muratani
Togay Oztaskin
Molly R. Perkins
Thomas A. Pologruto
Evan E. Santo
Ashish Saxena
Noriko Simorowski
Srinath Sridhar
Ji-Joon Song
Izabela Sujka
Zankhana Thakkar
Francisca von Saint Paul
Chia-Lin Wu

Postdoctoral Fellows

Jean-Raymond Ango
Andres Barria-Roman
Carine Becamel
Paula A. Bubulya
Hsu Hsin Chen
Debopriya Das
Rajdeep Das
Elisa De Stanchina
Robert Del Vecchio
Hiyuan Ding
Farida Emran
Peter J. Gillespie
Derek Goto
Eve Govek
Hui-Fu Guo
Susan M. Janicki
Cizhong Jiang
Natarajan Kannan
Carla E. Margulies
Jinrong Min
Pol O'Fearghail
Lance E. Palmer
Vijay Patankar
Amandine Penel
Emiliano Rial Verde
Jose L. Roig
Kalpana Samudre
Clare L. Scott
Gordon Shepherd
Pavel Sumazin
Shweta Tyagi
Vanisree Velamoor

The Postdoctoral Program at Cold Spring Harbor Laboratory

The Postdoctoral Program Office at Cold Spring Harbor Laboratory is continuing its efforts to meet the needs of the Laboratory's postdoctoral fellows (postdocs) and faculty, under the day-to-day manage-

ment of Alyson Kass-Eisler and the scientific leadership of Nicholas Tonks. This year, the Laboratory welcomed 31 new postdocs and saw 32 depart. The departing postdocs went on to positions at the Blanchette Rockefeller Neuroscience Institute, Duke University, Eli Lily and Company, GlaxoSmithKline, Hokkaido University, Lawrence Livermore National Laboratory, Northwestern University, The University of Oregon, Portland State University, Stony Brook University, Walter and Eliza Hall Institute of Medical Research, Washington University, The Wistar Institute, and Wright State University, to name but a few. We wish them every success for the future.

Postdocs at the Laboratory are encouraged to apply for individual fellowships to enhance their careers, as well as provide support for the Laboratory. The Postdoctoral Program Office provides our postdocs with an individualized list of eligible fellowships to which they can apply. These fellowships are highly competitive and require a great deal of work on the part of the applicant. We are very proud of our postdocs who have received these prestigious fellowships. In 2005, postdocs received awards from the following foundations: Alzheimer's Foundation, Army, Burroughs Wellcome Foundation, Canadian Institute of Health Research, Damon Runyon Cancer Research Foundation, FRAXA Foundation, Helen Hay Whitney Foundation, Hereditary Disease Foundation, Human Frontier of Science Program, Jane Coffin Childs Memorial Fund for Medical Research, Leukemia and Lymphoma Foundation, National Alliance for Research on Schizophrenia and Depression, National Institutes of Health, National Science Foundation, and Wellcome Trust.

Assisting postdocs with career development continues to be an important goal of the School. We were pleased to have been chosen in 2005 to participate in the Merck Research Laboratories Ambassador Recruiting Program. The goal of the program is to establish a long-standing relationship between the Merck Ambassador and the Laboratory's graduate students and postdoctoral trainees so that they can learn about potential career opportunities in industry directly from a Merck scientist. The "Ambassador" is Dr. Jing Li, a Research Fellow at MRL. In addition, MRL has provided a monetary gift to the graduate and postdoctoral programs to provide support for activities that promote scientific exchanges and career opportunity discussions among the trainees at the Laboratory. In one such lecture, David Vaux, Senior Principal Research Fellow in the Walter and Eliza Hall Institute's Molecular Genetics of Cancer Division, presented a lively lecture entitled "Ten rules of thumb for the presentation and interpretation of data in publications." In addition to the programs that we conduct here, the Laboratory's continuing partnership with the New York Academy of Science, Science Alliance has given our postdocs additional opportunities for networking and obtaining career development advice. This year, the Science Alliance seminar titles included:

- Faculty Search Committees: What Are They Looking For?
- Job Search Fundamentals
- Bioscience Research: Where Do You Fit In?: Naturejobs/NYAS Career Fair and Symposium

Postdoctoral trainees have had the opportunity to participate in teaching undergraduate and graduate students at the School. This year, participation has included course lectures, moderating discussion sessions in core courses, and student tutoring. Additionally, postdocs at the Laboratory gave lectures in the Undergraduate Research Program's Bioinformatics Workshops. We are extremely grateful for the expertise our postdocs have been able to share with the School's other educational programs.

To complement our local efforts, we have been involved on a national level in workshops aimed at shaping the postdoctoral experience. In 2004, we became founding members of the National Postdoc Association (NPA). In March 2005, Alyson Kass-Eisler attended the third annual meeting in San Diego, California. Topics of interest to the Laboratory's postdocs included:

- The NPA's Agenda for Change
- The Sigma Xi Postdoc Survey
- An overview of the NIH K Awards Program
- Career and Job Search Strategies for Postdocs
- Trends in Postdoctoral Training, Evaluating Best Practices

- Effective Strategies for Enhancing the Postdoctoral Experience
- Postdoc Policy 101
- A Comprehensive Pilot Mentoring Program
- Policy Roundtable: Fostering Collaboration
- Postdoc Work Visa Options
- Academic-Industry Relations
- Keynote Address by Steven Sample (President, University of Southern California)

Executive Committee

A large measure of the Watson School's success can be traced to the sage advice, guidance, and governance of the School's Executive Committee. January 2005 saw the induction of a new faculty member Terri Grodzicker, replacing Jan A. Witkowski who had faithfully served for 6 years. As happens each year, there was also turnover among the student representatives. The Watson School representative Darren Burgess was replaced by Hiroki Asari, and the Stony Brook University representative Marlies Rossmann was replaced by Despina Siolas. The School is indeed thankful for their frank, honest, and thoughtful advice.

Special Events: The Gavin Borden Fellow

The annual Gavin Borden Visiting Fellow (so named after the energetic and charismatic publisher of *Molecular Biology of the Cell*, who died of cancer in 1991) brings to the Laboratory an eminent researcher and educator to give the Gavin Borden Lecture, dedicated to the graduate students at the Laboratory. Dr. Huda Zoghbi from the Baylor College of Medicine and an Investigator with the Howard Hughes Medical Institute, as well as a practicing clinician, was this year's Fellow. Her lecture "*Cross-species Studies to Unfold the Pathogenesis of a Neurodegenerative Disease*" was thoroughly enjoyed as evidenced by the standing-room-only audience. In addition, Huda shared her experiences as a scientist and clinician at a roundtable discussion with the students the following day.

The Watson School Continues to Benefit from Generous Benefactors

For the last 5 years, the School has been the recipient of a grant from the Arnold and Mabel Beckman Foundation. Ms. Jacqueline Dorrance, Executive Director of the Beckman Foundation, made a site visit to the School in November 2004. Following on from her very successful visit and meetings with the students and faculty, in April 2005, we were invited to submit an application for renewed funding. I am extremely pleased to report that we have been awarded a second grant of $1.75 million from the Beckman Foundation! This 5-year grant will fund five students per year and contribute $100,000 annually toward the core courses. I would like to take this opportunity to thank Kiryn Haslinger from the Laboratory's Development Office for her superb assistance in preparing the funding application. In addition, we were notified in April that the School's National Institutes of Health training grant has been renewed for another year; we have again been awarded five slots. This spring, the School will submit a competitive renewal for this grant.

Eva Radeck

The Watson School Administration

The Watson School administration (a.k.a. Team Watson!) continues to grow from strength to strength. I am pleased to announce that Eva Radeck joined us as the School's administrative assistant in April. Eva took over from Mark Beavers who moved to Meetings and Courses earlier this year. Eva is a native of Germany, who studied

Beth and Madeleine

administration and foreign languages (English, French, and Spanish). Before joining the Laboratory, she worked for 7 years at the European Molecular Biology Laboratory in Heidelberg.

The WSBS Family Continues to Grow

On July 15, 2005, Beth Chen, of the entering class of 2002, and her husband, Pavel, welcomed a beautiful baby daughter, Madeleine Ekaterina, who weighed in at 7 pounds, 7 ounces. All are thriving!

This year has also seen several engagements. Shraddha Pai (from the entering class of 2004) announced her engagement to Lincoln Stein, and David Simpson (also from the entering class of 2004) announced his engagement to Erin Dann. And our very own "Team Watson" member, Dawn Meehan, became engaged to former graduate student Thomas Pologruto in February. Our congratulations and best wishes go to all of them!

Lilian Clark Gann
Dean

SPRING CURRICULUM

TOPICS IN BIOLOGY

ARRANGED BY **Dawn Meehan**
Alyson Kass-Eisler
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 instructor or a team of invited instructors offer 7-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 2005, there were two such courses: *Human Behavior* and *Evolution*.

Human Behavior

Attended by the entering classes of 2001 and 2002

INSTRUCTOR **Jill M. Mateo**, University of Chicago

GUEST LECTURER **Michael J. Owren**, Cornell University

TEACHING FELLOW **Kara Nuss**, University of Chicago

(*Left to right, kneeling*) Jonathan Kui, Shu-Ling Chiu, Allison Blum. (*Left to right, standing*) Beth Chen, Claudia Feierstein, Tomas Hromadka, Gowan Tervo, Charles Kopec, Elizabeth Murchison, Catherine Cormier, Kara Nuss, Darren Burgess, Jill Mateo

The essence of this course was a radically different way of thinking about why animals, including humans, behave as they do. In contrast to physiological, developmental, cognitive, or other "proximate" approaches to behavior, in this course, evolutionary or functional approaches were primarily used. The question repeatedly asked was "How does a particular behavior contribute ultimately to the survival and reproductive success of an individual exhibiting the behavior?" A shorthand of this is "What is the adaptive significance of the behavior?" Topics in the course included an overview of Darwin's theory of evolution by natural selection, inclusive fitness, kin selection, and the evolution of altruism, genetic-developmental influences on behavior, the evolution of sexual reproduction and of the sex ratio, the evolution of mating systems and sexual selection, and the evolution of male and female reproductive strategies, among others. Note that most of these behaviors are social behaviors. The greatest proportion of attention was given to animals other than humans, but with the intention of understanding the behavior of all animal species, including humans, in an evolutionary framework. The course revolved around Darwin's theory of organic evolution by the process of natural selection. This theory and some of its recent extensions were used to examine animal behavior in an evolutionary context. The emphasis of course content was on concepts, ideas, and theories, which were described and clarified with specific examples from many animal species.

The course ran from Sunday to Saturday (March 28–April 3) and was organized and largely taught by Jill Mateo. It integrated lectures by the instructors, daily quizzes, directed readings of research papers, seminars by the instructors, and a field trip to the Bronx Zoo. The students, many of whom had little prior awareness of the subject, rated the course very highly.

Evolution

Attended by the entering classes of 2003 and 2004

INSTRUCTOR **Nipam H. Patel**, University of California, Berkeley

GUEST LECTURERS **Casey Bergman**, Cambridge University, United Kingdom
Rob DeSalle, American Museum of Natural History
Neil Shubin, University of Chicago

TEACHING FELLOWS **Brian Kraatz**, University of California, Berkeley
Danielle Liubicich, University of California, Berkeley
John Novembre, University of California, Berkeley

The field of evolutionary biology touches upon all other areas of the biological sciences, since every form of life and every biological process represent an ongoing evolutionary "experiment." The aim of this course was both to discuss the understanding of the mechanisms of evolution and to explore how evolutionary data can be used to further the understanding of various biological problems.

The course began with a discussion of the diversity of organisms that currently exist and our methods for understanding the evolutionary relationships among these organisms. It went on to study how paleontological data are collected and used to understand the history of life on earth and then examined how DNA sequence data can be used to understand the evolutionary history of organisms, genes, and genomes. Within this molecular and genetic framework, the focus shifted to the mechanisms of evolutionary change and how variation within populations leads to the evolution of new species. Finally, there were discussions about how morphological changes are brought about through evolutionary changes in development and gene regulation. The hope was that this course would provide the student with a general overview of evolutionary biology and how to use evolutionary data to gain further insight into all manner of biological problems.

(*Left to right*) John Novembre, Brian Kraatz, Shraddha Pai, Keisha John, Oliver Fregoso, Daniel Chitwood, Izabela Sujka, Molly Perkins, Casey Bergman, David Simpson, Galen Collins, Nipam H. Patel, Monica Dus, Hiroki Asari, Francois Bolduc, Wei Wei, Christopher Harvey, Evan Santo, Danielle Liubicich

The course ran from Sunday to Saturday (April 17–April 23) and was organized and largely taught by Nipam H. Patel. It integrated lectures by the instructors, directed readings of research papers, seminars by the instructors and guest lecturers, and a creation of a "tree of life" based on their hands-on study of living biological samples. As in previous years, the course was highly rated by all of the students.

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Craig Hinkley (Lead Instructor, High School)**
Erin Maroney (Lead Instructor, Middle School)
Athanasios (Tom) Bubulya
Elna Carasco
Jeanette Collette
Uwe Hilgert
Amanda McBrien

ADMINISTRATOR **Carolyn Reid**

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 and members of the School's Executive Committee attend the talks and give individual feedback to students on their presentations. This year, 17 faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS

Carlos D. Brody
Josh Dubnau
Leemor Joshua-Tor
Eli Hatchwell
Scott Lowe
Zachary Mainen
Alea A. Mills
Senthil K. Muthuswamy
Michael P. Myers
Scott Powers
David L. Spector
Lincoln Stein
William Tansey
Marja Timmermans
Nicholas Tonks
Linda Van Aelst
Michael Q. Zhang

FALL COURSE CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

FUNDED IN PART BY **The Arnold and Mabel Beckman Foundation; The William Stamps Farish Lectureship**

INSTRUCTORS
Senthil K. Muthuswamy (Lead)
Gregory Hannon
Z. Josh Huang
W. Richard McCombie
Scott Powers
Marja Timmermans

GUEST LECTURERS
Grigori Enikolopov
Leemor Joshua-Tor
Adrian R. Krainer
Scott Lowe
Wolfgang Lukowitz
Michael P. Myers
Patrick J. Paddison
Cordula Schulz
William Tansey
Nicholas Tonks
Bruce Stillman

VISITING LECTURER **William C. Merrick,** Case Western Reserve University

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 six 2-week modules, each of which had a different theme. For each module, 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 among themselves to discuss assigned papers not covered by the problem set. At the end of each week, the students spent the evening discussing these papers with faculty. In the final module, Study Section, the students participated in a mock review of real, funded Federal R01 grants including oral presentations and written critiques.

Module 1	Gene Expression	Module 4	Development
Module 2	Cell Division Cycle	Module 5	Genomics and Proteomics
Module 3	Signal Transduction	Module 6	Study Section

The Darrell Core Course on Scientific Exposition and Ethics

FUNDED IN PART BY **The Arnold and Mabel 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)
Yuri Lazebnik
Jan A. Witkowski

VISITING LECTURERS
Robert P. Charrow, Greenberg Traurig LLP
Colin Goddard, OSI Pharmaceuticals
Peter Petre, Fortune Magazine
Philip Reilly, Interleukin Genetics
Bodo Stern, Cell Press
David Vaux, Walter and Eliza Hall Institute

This core course offered instruction in 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. Writing skills included the fundamentals of modern scientific English and the organization and preparation of papers, research abstracts, and grant applications. Oral presentation skills were taught by scientists with excellent, albeit different, modes of presentation. Together with instructors, students critiqued formal seminar presentations at the Laboratory. Instruction and discussions about ethics included the ethical implications of biological discovery on society (e.g., the implications of the cloned sheep Dolly for human cloning) as well as the nature and boundaries of ethical behavior of scientists and their rights and responsibilities. A primary objective of the course was that students consider exposition and ethics an integral part of scientific research.

Research Topics

ARRANGED BY
Lilian Clark Gann
Eva Radeck

This core course provided students with an in-depth introduction to the fields of research that 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 Carlos D. Brody, Hollis Cline, Grigori Enikolopov
Week 2 Gregory Hannon, Z. Josh Huang, David Jackson
Week 3 Leemor Joshua-Tor, Alexei Koulakov, Adrian R. Krainer
Week 4 Scott Lowe, Yuri Lazebnik, Robert Lucito
Week 5 Wolfgang Lukowitz, Zachary Mainen, Robert Martienssen

Week 6	W. Richard McCombie, Alea A. Mills, Vivek Mittal
Week 7	Senthil K. Muthuswamy, Michael P. Myers, Jonathan Sebat
Week 8	David L. Spector, Lincoln Stein, Arne Stenlund
Week 9	Bruce Stillman, William Tansey, Marja Timmermans
Week 10	Linda Van Aelst, Michael Wigler, Anthony Zador
Week 11	Michael Q. Zhang, Yi Zhong

SPECIALIZED DISCIPLINES COURSES

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 (Lead)**
Josh Dubnau
Lincoln Stein

GUEST LECTURER **Wolfgang Lukowitz**

VISITING LECTURER **Bambos Kyriacou,** University of Leicester, United Kingdom

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 can be integrated (1) "vertically" to understand how molecular mechanisms influence functional output from various levels of biological organization and (2) "horizontally" to understand how genetic pathways have been conserved evolutionarily.

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 help to define biological processes? How are more complex traits genetically dissected into simpler (underlying) 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 is it 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.

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 (Lead)** **Linda Van Aelst**
VISITING LECTURERS	**Michael Caplan,** Yale University **Gregg Gundersen,** Columbia University **Conly Rieder,** Wadsworth Center, New York State Department of Health **Marc Symons,** North Shore–LIJ Research Institute **Graham Warren,** Yale University

With the complete genome sequence available for many organisms, there is now an increasing emphasis on understanding the function of the gene products. This understanding requires an increasing appreciation of the structure and function of the cell as well as dynamic associations within the cell. This course provided a basic overview of the structural and functional organization of cells with particular emphasis on cellular compartmentalization and communication. Topics of focus included the cytoskeleton, cell adhesion and signaling, membrane transport, gene expression, and nuclear organization. In addition, the course provided insight into the basic toolbox of the cell biologist of the 21st century.

Neuroscience: Systems and Behavior

FUNDED IN PART BY	**The George W. Cutting Lectureship; The Klingenstein Lectureship**
INSTRUCTORS	**Carlos D. Brody (Lead)** **Zachary Mainen**
GUEST INSTRUCTORS	**Dmitri Chklovskii** **Roberto Malinow**

This course introduced students to neuroscience, with a focus on systems and behavior. No prior knowledge of neuroscience was assumed. The course began by describing the basics of electrical signaling in neurons: membrane potentials, ion channels, action potentials, and synaptic transmission. How do such building blocks translate into whole-organism behavior? The instructors first discussed classical conditioning and asked how changes insynaptic transmission could underlie such behavior. They then discussed associative learning, Hebb's postulates, LTP (long-term synaptic potentiation), and some computational models of associative learning. From behaviors that focus on simple memories, they turned to behaviors that require making perceptual decisions. Some basic concepts of perceptual neuroscience, such as neuronal "receptive fields," were covered and then used to discuss current results and models of perceptual decision making. Finally, they turned to the learning of temporal sequences and discussed the temporal credit assignment problem, dopamine and VTA neuron responses, temporal difference models of learning, and addictive behavior.

UNDERGRADUATE RESEARCH PROGRAM

Program Directors: David Jackson
Lincoln Stein

Program Administrator: Dawn Meehan

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, 676 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 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 15-minute seminar describing the background, design, and results of the experiments during the course of a 2-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 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 597 applicants, took part in the 2005 program:

(*Top row, left to right*) Alexei Finski, Wei Gan, Alexandra Nica, Victoria Svinti, Tasleem Samji, Christian Sanchez-Jordan, Albert Almada, Dailia Francis, Jonathan Chen, Margot Rommens, Laura Wherity, Kelly Wetmore. (*Bottom row, left to right*) David Wurtz, Robert Carrasquillo, Krishnan Palaniappan, Marek Kudla, Vanessa Ringgold, Scott Millman, Nora Seidl, Yaniv Erlich, Ye Wang, Christopher Javadi, Vineeta Argawala, Thomas Takara, Betty Kong

Vineeta Agarwala, Stanford University
Advisor: **Dr. Michael Q. Zhang**
Sponsor: National Science Foundation
CTCF binding-site specificity and distribution.

Albert Almada, University of California, Irvine
Advisor: **Dr. Andrew Neuwald**
Sponsor: National Science Foundation
Exploring the relationship between sequence, structure, and function in the αβ hydrolase fold family.

Robert Carrasquillo, Washington University, Saint Louis
Advisor: **Dr. Robert Martienssen**
Sponsor: National Science Foundation
Effects of differential methylation on transposon activation and gene expression in *A. thaliana.*

Jonathan Chen, Oberlin College
Advisor: **Dr. Gregory Hannon**
Sponsor: Burroughs-Wellcome Fund
Utilizing RNAi to identify metastasis-associated genes.

Yaniv Erlich, Tel-Aviv University
Advisor: **Dr. Partha P. Mitra**
Sponsor: Emanuel Ax Fund
Novel wireless sensor network for electrophysiology and behavioral research.

Alexie Finski, International University, Bremen
Advisor: **Dr. Zachary Mainen**
Sponsor: H. Bentley Glass
Two-photon imaging of spines and cell populations in head-fixed awake behaving animals.

Dailia Francis, Hunter College
Advisor: **Dr. Alea A. Mills**
Sponsors: William Townsend Porter Foundation and Hunter College
Novel tumor suppressor gene(s) at human 1p36.

Wei Kevin Gan, Harvard College
Advisor: **Dr. William Tansey**
Sponsor: Olney Foundation
Exploring the role of the 20S proteasome subunit in transcription regulation.

Christopher Javadi, University of Texas, Austin
Advisor: **Dr. Josh Dubnau**
Sponsor: National Science Foundation
Drosophila deficiency mapping using whole-genome tiling arrays.

Betty Kong, Rutgers University
Advisor: **Dr. Vivek Mittal**
Sponsor: Joan Redmond Read Fund
Developing an in vitro assay for studying the function of bone-marrow-derived lineage-depleted cells in vasculature formation.

Marek Kudla, Warsaw University
Advisor: **Dr. Rui-Ming Xu**
Sponsor: Former URP Fund
Prp8: The elusive structure of a crucial spliceosomal component.

Scott Millman, Cornell University
Advisor: **Dr. Adrian R. Krainer**
Sponsor: Joe Lewis Jefferson Foundation
Mutational analysis of the oncogenic activity of SF2/ASF.

Alexandra Nica, International University, Bremen
Advisor: **Dr. W. Richard McCombie**
Sponsor: Former URP Fund
Genome-wide SNP detection in *Oryza sativa* strains using a massively parallel sequencing strategy.

Krishnan Palaniappan, Carnegie Mellon University
Advisor: **Dr. Bruce Stillman**
Sponsor: National Science Foundation
Binding of mitotic cyclins to Cdc6 and ORC as regulators of prereplication complex formation.

Vanessa Ringgold, University of California, Davis
Advisor: **Dr. Marja Timmermans**
Sponsor: National Science Foundation
Investigations into the affects of ASYMMETRIC LEAVES 1 in *Arabidopsis.*

Margot Rommens, University of Leuven
Advisor: **Dr. Linda Van Aelst**
Sponsor: Jephson Educational Trust
Oligophrenin: Where art thou? Detecting OPHN-specific phage clones for subsequent germ-line manipulation in mice.

Tasleem Samji, Cambridge University
Advisor: **Dr. Senthil K. Muthuswamy**
Sponsor: C. Bliss Memorial Fund
Silencing Par6a in breast epithelial cell lines.

Christian Sanchez-Jordan, Johns Hopkins University
Advisor: **Dr. Robert Lucito**
Sponsor: Garfield Fellowship
High-throughput RT-qPCR: Narrowing the list of candidate tumor suppressor genes in ovarian and other cancers.

Nora Seidl, Cambridge University
Advisor: **Dr. David Jackson**
Sponsor: Von Stade Fellowship
Isolation of FEA2 and associated proteins.

Victoria Svinti, Nui Maynooth, Ireland
Advisor: **Dr. Lincoln Stein**
Sponsor: Libby Fellowship
Programmed frameshifts in *Paramecium*.

Thomas Takara, Grinell University
Advisor: **Dr. Leemor Joshua-Tor**
Sponsor: National Science Foundation
A structural investigation of papillomavirus replication initiation protein E1.

Ye Wang, University of Rochester
Advisor: **Dr. Cordula Shultz**
Sponsor: National Science Foundation
ppd—To be stem cells, or not to be.

Kelly Wetmore, University of California, Los Angeles
Advisor: **Dr. Wolfgang Lukowitz**
Sponsors: Steamboat Foundation and the National Science Foundation
Mapping quantitative trait loci that modify mutations in SHORT SUSPENSOR, a predicted kinase regulating plant embryogenesis.

Laura Wherity, Oxford University
Advisor: **Dr. Alexei Koulakov**
Sponsor: William Shakespeare Fellowship
Obtaining graded values of synaptic strength in the CaMKII and PP1 feedback loop in neurons.

David Wurtz, Olin College of Engineering
Advisor: **Dr. Yuri Lazebnik**
Sponsor: Jephson Educational Trust
The cause and effect of multinucleation.

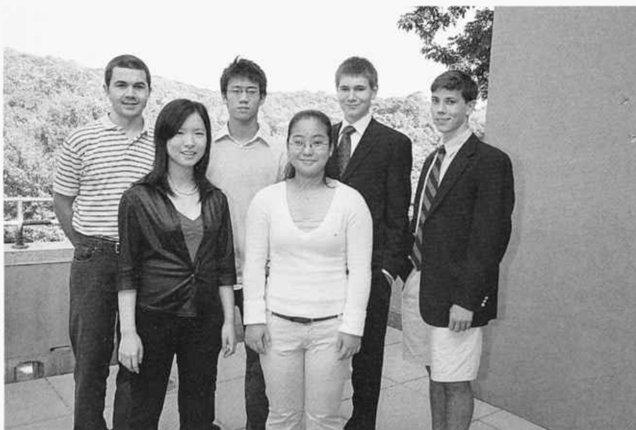
PARTNERS FOR THE FUTURE

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. The six students selected spend a minimum of 10 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 students give oral presentations of their research projects to an enthusiastic audience of the students' scientific mentors, Lab administrators, parents, and teachers. Although the students learn a great deal about modern 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 2005–2006 Partners for the Future are:

Partner	High School	CSHL Mentor	Laboratory
Daniel Capurso	Sayville High School	Anindya Bagchi	Alea A. Mills
Faith Chang	Syosset High School	Vivek Mittal	Robert Martienssen
Yuting Chiang	The Wheatley School	Cordula Schulz	
James Gurtowski	Cold Spring Harbor High School	Ravi Sachidanandam	
Yuri Hanada	Farmingdale High School	Eli Hatchwell	
George Roche	Cold Spring Harbor High School	Matthew Vaughn	Robert Martienssen



(*Left to right*) Daniel Capurso, Faith Chang, Yuting Chiang, Yuri Hanada, James Gurtowski, George Roche

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and secondary school students the opportunity to acquire 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 2005, 321 students participated in 31 courses within the program. The classes were held outdoors, weather permitting, at the Southdown School where the Laboratory has equipped and maintains classroom and laboratory facilities. This facility is used as a base for the student's 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 2-week sessions, the *Adventure Education* course met again on July 15, 2005 when we chartered the Schooner Phoenix for an exciting environmental education program. Living samples of the fauna of Long Island Sound were collected by using the Otter Trawl. The children were encouraged to hold and touch the sea life as part of their experience. Sea Stars and horseshoe crabs were of particular interest. We did experiments measuring the depth of the waters we were in, the salinity, and temperature. Pollution and the cause and effects on the animals and the water were discussed. We also used a microscope to examine plankton and other microorganisms. After we motored out to the sound, the children were shown how to raise and lower the sails.

On July 22, 2005, *Adventure Education* went on a 6-mile canoe trip on the Nissequogue River in Smithtown to navigate and explore the waters of Long Island. The course emphasized the plant and animal life indigenous to the area as well as historic points of interest.

This year, we had *Nature Photography I* and *II*. The courses focused on taking pictures out in the field as well as studio shots. A darkroom was set up so that the students could develop and print their own black and white film, as well as experiment with different photographic techniques. Students in *Photography II* were introduced to digital photography. One of their projects was to make a photo collage of the pictures they took. We set up a computer and a color printer as well as the dark room so that the children could always be developing their skills.

PROGRAM DIRECTOR: William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR: Sharon Bense, Cold Spring Harbor Laboratory

INSTRUCTORS: 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

COURSES

Nature Bugs (Kindergarten): Exploration, games, stories, and dramatics are used to introduce the young child to a 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 a succession of communities are studied. Students study the diversity of plant and animal forms native to the Cold Spring Harbor Laboratory 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): 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.

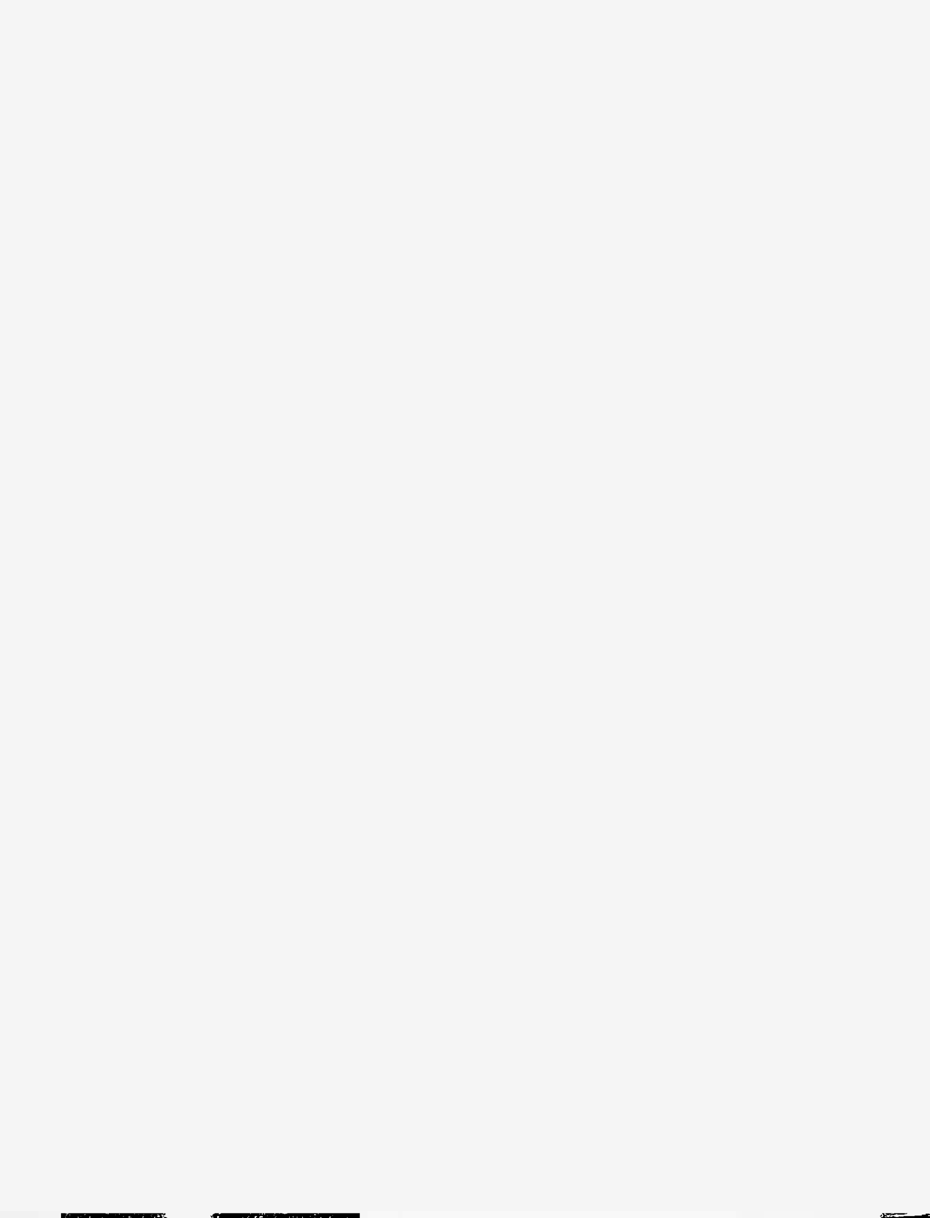
Freshwater Life (Grades 5–7): Students study the vertebrate, invertebrate, and plant life found in area bogs, ponds, lakes, and streams.

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 (A) Schooner Phoenix (Grades 5–7): Students join the Nature Study staff for a 4-hour sail on board the *Phoenix* (a 71-foot schooner), where they identify some of the animals brought up in the ship's trawl, learn how to navigate, and see how this tall ship operates from stem to stern (the *Phoenix* is a USCG-certified vessel) .

Adventure Education (B) Canoe Trip (Grade 6–10): This course is a 6-mile canoe trip up and down the Nissequouge 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. It 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 2005, 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 *DNA Microarray Applications* was twinned with a new course on *Statistics of Microarray and Related Biological Data* because the majority of investigators are increasingly interested in the experimental design and analysis of the large data sets generated by these kinds of experiments.

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 2005 courses and instructors is listed on the following pages.

Grants from a variety of sources support the courses. These include multiple awards from the National Institutes of Health (NIH) and the National Science Foundation (NSF). We also have a valuable large education grant from the Howard Hughes Medical Institute for the support of neurobiology courses and new courses. The courses also depend on equipment and reagents that are loaned or donated by a large number of companies, which are invaluable for making it possible to keep up with the latest technologies.

The Laboratory held 22 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.

As often happens, many of this year's meetings were oversubscribed, including *The Biology of Genomes, Retroviruses, Mechanisms of Eukaryotic Transcription*, and *Neurobiology of* Drosophila, as well as the annual Symposium. 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 NIH, NSF, 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—now in its 70th year—continues to be the flagship conference of the meetings program. This year's meeting on "Molecular Approaches to Controlling Cancer" addressed many aspects of cancer research and featured 72 talks and 240 poster presentations. Opening night speakers included Dennis Slamon, Harold Varmus, Mina Bissell, and James Allison, and Tyler Jacks gave a very eloquent summary. Titia de Lange presented the Reginald Harris lecture on "Protection of Human Telomeres." Charles Sawyers gave the Dorcas Cummings lecture on "Making Progress through Molecular Attacks on Cancer" to a mixed audience of scientists and lay friends and neighbors of the laboratory.

J. Watson and S. Gottesman at *Advanced Bacterial Genetics 60th Anniversary Symposium*

The joint Cold Spring Harbor/Wellcome Trust conference series held at the genome campus south of Cambridge, England, was expanded to include meetings on *Interactome Networks, Prion Biology*, and *Functional Genomics of Mammalian Nervous Systems*. These conferences follow the Cold Spring Harbor model in that the majority of talks are selected from the abstracts.

The success of the very large number of meetings and courses is also due to the skilled work of many Cold Spring Harbor Laboratory staff and faculty who contribute their expertise, efforts, and good humor to the program.

Terri Grodzicker
Assistant Director
for Academic Affairs

David Stewart
Executive Director,
Meetings and Course

Advanced Bacterial Genetics 60th Anniversary Symposium pool party at Banbury Center

Searching for clues in the Courses scavenger hunt

The Courses Scavenger Hunt winning team members of the *Proteomics* course

The plate race

Attendees at the *Symposium to Celebrate the Scientific Explorations of Walter Gilbert*

Walter (Wally) Gilbert at *Tasting The Art of Science Symposium to Celebrate the Scientific Explorations of Walter Gilbert*

70TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Molecular Approaches to Controlling Cancer

June 1–6 515 participants

ARRANGED BY **Bruce Stillman and David Stewart,** Cold Spring Harbor Laboratory

During the past quarter century or so, much effort has been devoted to understanding the molecular basis of cancer. We now know that cancer is primarily a genetic disease of mutations in the tumor genome that is acquired over a lifetime. The products of oncogenes and tumor suppressor genes have been placed into pathways of gene networks that are altered in tumor cells, compared to normal cells in tissue. Furthermore, we also understand that tumors function as abnormal organs, forming an architecture of a number of different cell types and recruiting a blood supply, albeit an irregular one. Many Symposia in this series have dealt with cancer directly and even more have focused on basic biology. These have contributed greatly to understanding cancer.

As a result of some interesting developments in cancer diagnosis and therapy during the past 5 years, it was appropriate that a Symposium be devoted for the first time to molecular approaches to cancer therapy. Several examples now exist of targeted therapy that works in patients who have been profiled on the basis of the genetic diagnosis of the patient's tumor. Additionally, therapies targeting the tumor as an organ, such as anti-angiogenic therapy, are now in the clinic with modest success. The hope is that this type of molecular approach to cancer therapy will accelerate and become more effective in the future.

In organizing this Symposium with help from Terri Grodzicker, we relied on the assistance of a number of colleagues including David Livingston, Craig Thompson, and Scott Lowe for suggestions on the choice of speakers. We thank them for their valuable advice. We also wish to thank the first evening speakers, Dennis Slamon, Harold Varmus, Mina Bissell, and Jim Allison, for providing an overview of the areas to be covered. This year's Reginald Harris Lecture was delivered by Titia de Lange on telomere biology and genomic stability. We particularly wish to thank Tyler Jacks for delivering a thoughtful and realistic summary of the progress we have made toward reaching our goal. Charles Sawyers, who conveyed the excitement that many of us feel that some inroads into treating cancer will be made by targeted therapies, presented the Dorcas Cummings Lecture to the local community and attending scientists. The effort put into the Dorcas Cummings Lecture by Charles and the summary by Tyler were highlights of the meeting.

B. Clarkson, B. Stillman

This Symposium was attended by 515 scientists and clinicians, and the program included 71 oral presentations and 239 poster presentations. A discussion, chaired by Bruce Alberts, discussed the proposal to analyze the genomes of all major human cancers to advance cancer diagnosis and therapy approaches.

Essential funds to run this meeting were obtained from the National Cancer Institute, a branch of the National Institutes of Health. 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 include Amgen Inc.; Bristol-Myers Squibb Company; GlaxoSmithKline; Novartis Institutes for

BioMedical Research. Corporate Sponsors include Applied Biosystems; AstraZeneca; BioVentures, Inc.; 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 include ArborGen; Monsanto Company. Corporate Affiliates include Affymetrix, Inc.; Agencourt Biosciences Corporation. Corporate Contributors include Aviva Systems Biology; Biogen, Inc.; EMD Bioscience; Illumina; IRx Therapeutics; Qiagen. Foundations include Albert B. Sabin Vaccine Institute, Inc.; Hudson Alpha Institute for Biotechnology.

R. Bernards, T. Grodzicker, E. Harlow

Cancer Genome Project Discussion Panel: Eric Lander, Sydney Brenner, Harold Varmus, Bruce Alberts, Joan Brugge, June Peterson, Anne Barker

R. Kalluri, J. Watson, D. Hanahan

Corporate sponsor wine and cheese party by the gazebo

D. Livingston, B. Ponder, M.-C. King

PROGRAM

Introduction
B. Stillman, *Cold Spring Harbor Laboratory*

DNA Damage Response and Cancer Genetics
Chairperson: D. Lane, *University of Dundee, United Kingdom*

Cancer Genetics and Genomes
Chairperson: J. Brugge, *Harvard Medical School, Boston, Massachusetts*

Tumor Responses to Microenvironment
Chairperson: C. Greider, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Cancer Genome Project Discussion
Chairperson: Bruce Alberts, *National Academy of Sciences, Washington, D.C.*

Reginald G. Harris Lecture: Protection of Human Telomeres
Chairperson: T. de Lange, *The Rockefeller University, New York*

Telomeres, Senescence, and Aging
Chairperson: S. Lowe, *Cold Spring Harbor Laboratory*

Animal Models for Cancer
Chairperson: M. Tessier-Lavigne, *Genentech Inc., South San Francisco, California*

Gene Expression and Cancer
Chairperson: J.M. Bishop, *University of California, San Francisco*

Dorcas Cummings Lecture: Making Progress through Molecular Attacks on Cancer
Speaker: Charles Sawyer, *University of California, Los Angeles*

Discovering Cancer Targets
Chairperson: M. Barbacid, *Centro Nacional de Investigaciones Ontologica, Madrid, Spain*

Biology of Cancer Cells and Tissues
Chairperson: C. Sherr, *Howard Hughes Medical Institute, St. Jude Children's Research Hospital, Memphis, Tennessee*

Stem Cells, Cancer Genes, and Therapeutic Approaches
Chairperson: J. Lees, *Massachusetts Institute of Technology, Cambridge*

More Therapeutic Approaches
Chairperson: B. Ponder, *University of Cambridge, United Kingdom*

Summary
Tyler Jacks, *Howard Hughes Medical Institute, Massachusetts Institute of Technology*

Grace lobby after the Dorcas Cummings lecture

MEETINGS

Imaging Neurons and Neural Activity: New Methods, New Results

March 10–13 236 participants

ARRANGED BY **Atsushi Miyawaki,** RIKEN Brain Science Institute
Joshua Sanes, Harvard University
Karel Svoboda, Cold Spring Harbor Laboratory

This new Cold Spring Harbor winter conference focused on imaging neuronal structure and function using a variety of advanced techniques, including a wide range of light microscopy techniques, electron microscopy approaches, and magnetic resonance imaging. It also explored emerging methodologies for manipulating neuronal function with a particular emphasis on genetics and chemistry. This unique forum was designed to stimulate future research studies in this important area and to promote interactions among researchers in diverse basic scientific areas relevant to this field. Roger Tsien delivered a fascinating keynote address on "Imaging Neuronal Protein Synthesis and Signaling." The majority of the talks were chosen from openly submitted abstracts. In all, this new conference attracted 127 abstracts, and progress in the field was summarized in thoughtful concluding remarks by Atsushi Miyawaki. The general consensus of participants was that the meeting should be held again at Cold Spring Harbor in 2007.

Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations provided core support for this meeting.

G. Rubin, D. Stewart, J. Huang

Introductory Remarks and Keynote Address: Imaging Neuronal Protein Synthesis and Signaling
K. Svoboda, *Cold Spring Harbor Laboratory*; R. Tsien, *Howard Hughes Medical Institute. University of California, San Diego*

Probing Connectivity
Chairpersons: L. Luo, *Stanford University, California*; C. Dulac, *Howard Hughes Medical Institute. Harvard University, Cambridge, Massachusetts*

New Imaging Modalities I
Chairpersons: W. Denk, *Max-Planck Institute for Medical Research, Heidelberg, Germany*; S. Dymecki, *Harvard Medical School, Boston, Massachusetts*

New Imaging Modalities II
Chairpersons: A. Konnerth, *Ludwig-Maximilians-University, Munich, Germany*; H. Monyer, *University of Heidelberg, Germany*

Imaging Cellular Structure and Function
Chairpersons: R. Wong, *Washington University, St. Louis, Missouri*; S. Fraser, *California Institute of Technology, Pasadena*

Assaying and Manipulating Neuronal Activity
Chairpersons: E. Callaway, *Salk Institute for Biological Studies, La Jolla, California*; G. Miesenboeck, *Yale University, New Haven, Connecticut*

Fluorescent Reporters and Other Labeling Strategies
Chairpersons: E. Isacoff, *University of California, Berkeley*; O. Griesbeck, *Max-Planck Institute of Neurobiology, Martinsried, Germany*

Concluding Remarks
Atsushi Miyawaki, *RIKEN Brain Science Institute*

K. Svoboda, E. Nedivi

B. Salzberg, W. Webb

K. Ibaraki, M. Nishi, M. Zlatic

Systems Biology: Global Regulation of Gene Expression

March 17–20 207 participants

ARRANGED BY **Jack Keene,** Duke University Medical Center
Peggy Farnham, University of California, Davis
Saeed Tavazoie, Princeton University

Gene expression is one of the most important processes determining the functional characteristics of organisms. Investigating the mechanisms that regulate gene expression is essential to understanding all biological processes and diseases. Most knowledge of the regulation of gene expression in the past has been derived from investigations of single interactions and outcomes. However, the onset of the genomic revolution has allowed for the first time the ability to investigate many events in gene expression simultaneously and in parallel. Much progress has been made in the study of transcriptional regulation, but the global regulation of gene expression involving posttranscriptional mechanisms as well is especially challenging. Therefore, the global analysis of gene expression involving both transcription and posttranscription was the subject of this third meeting on Systems Biology. It was thus unique in that it brought together researchers using three major approaches to global gene expression and systems biology, including transcription, posttranscription (RNA splicing, localization, stability, and translation), and computational analysis. The sessions were structured with an opening keynote speaker whose research overlaps all of these three areas and was followed in the first session by representative presentations from each of the three areas. The subsequent sessions considered first computational, then transcriptional, then posttranscriptional, and then comparative genomics, followed on the last day with a session on emerging technologies. A diverse poster session represented these three approaches to understanding global gene expression and provided direct interactions among investigators with these common interests. Many participants commented on the quality of the meeting and in a planning session, enthusiasm was expressed to continue this meeting series in its current format.

J. Keene, J. Witkowski, A. Krainer

A decision was made to include a tutorial prior to the formal meeting that would familiarize participants with computational methods relevant to transcriptional and posttranscriptional regulation of global gene expression.

Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations provided core support for this meeting.

PROGRAM

Keynote Address: Overture
R. Young, *Whitehead Institute for Biomedical Research, Cambridge, Massachusetts*

Computational Approaches to Identifying *Cis*-regulatory Elements
Chairperson: S. Tavazoie, *Princeton University, New Jersey*

Advances in Detection of Transcription Factor/DNA Interactions
Chairperson: P. Farnham, *University of California, Davis*

Transcriptional and Posttranscriptional Network Modeling
Chairperson: J. Keene, *Duke University Medical Center, Durham, North Carolina*

Comparative Genomics of Global Gene Regulation
Chairperson: A. Gasch, *University of Wisconsin, Madison*

Emerging Technologies and Concepts in Systems Biology
Chairperson: M. Bulyk, *Harvard Medical School, Boston, Massachusetts*

R. Young, D. Gifford, Y. Ruan

S. Sperling, J. Lohmann, W. Busch, D. Arnosti

S. West, T. Silkov, N. Nogoy

Learning and Memory

April 20–24 175 participants

ARRANGED BY **John Byrne,** University of Texas, Houston Medical School
Joseph LeDoux, New York University
Erin Schuman, HHMI, California Institute of Technology

This year's meeting tackled the issue of plasticity on many levels, ranging from neurogenesis to behavioral memory mechanisms. Presenters in the neurogenesis session discussed the effects of the environment and NMDA receptor activity on new neuron survival, as well as the contribution of neurogenesis to some forms of learning. Two talks in a plasticity session reemphasized the importance of intrinsic excitability changes, rather than synaptic changes, in bringing about plasticity at the level of neural circuits and behavior. Switch-like mnemonic firing patterns of entorhinal cortical neurons were also described. Other talks emphasized neurotransmitters and signaling pathways that contribute to long-term potentiation. In a session emphasizing structural plasticity, alterations in spines induced by the molecules β-catenin and Shank were discussed. In addition, the trafficking of polyribosomes into spines following LTP was also shown; spines with polyribosomes are enlarged. Imaging experiments demonstrated a relationship between the initial spine size and the facility with which plasticity can be induced: Small spines containing functional NMDA receptors and small numbers of functional AMPA receptors are more likely to show plasticity. One session highlighted local control of plasticity at the synapse. Topics such as regulation of local protein translation by miniature synaptic events and mGluRs were discussed. In addition, the importance of the specific pattern of synaptic input activation, and its location on the dendritic tree, was discussed. In the session dedicated to memory encoding in cortical circuits, the importance of θ rhythms for both semantic and episodic memory was discussed. The firing properties of a subset of medial entorhinal cortical cells were also described; these "grid cells" fire at specific intersection points in the animal's environment. In a session addressing the role of transcription factors in learning and memory, several presentations discussed the role of CaMKII as a molecular memory molecule. In addition, the role of CREB and IEG activation in memory formation was addressed. An epigenetic model for

E. Schuman, J. Byrne

T. Houpt, D. Lockwood, G. Golden

cellular memory was also presented. A session on the complexities of simple systems included several presentations addressing various molecular mechanisms underlying long-term memory, intermediate-term memory, and the differences between classical and operant conditioning in *Aplysia*. Other talks covered the role of Synaptotagmin 4 in retrograde signaling underlying synapse-specific potentiation and growth in the *Drosophila* neuromuscular junction, as well as the role of the dorsal paired medial neurons in the *Drosophila* mushroom body in olfactory memory. Finally, there were systems-level sessions on consolidation/reconsolidation and on extinction. Presentations in the consolidation/reconsolidation focused in particular on the roles of the prefrontal cortex and amygdala. One talk presented evidence that inhibition of protein synthesis in the mPFC following reactivation of a fear memory had no effect on memory, suggesting that reconsolidation does not occur in this structure. Another presentation examined the molecular cascades activated in the mPFC in extinction of conditioned fear and found evidence that similar molecular cascades are activated in both the amygdala and mPFC. A talk investigating the interaction between extinction and reconsolidation of fear memory in the amygdala indicated no competition between these two processes in the basal lateral amygdala. Finally, data were presented indicating that extinction acquisition depends on activation of the L-type voltage-gated calcium channel in the BLA and that extinction expression is mediated by increases of GABA(A) transmission in the BLA.

Major sponsorship for this meeting was provided 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 affiliates, corporate contributors, and foundations also provided core support for this meeting.

PROGRAM

Neurogenesis
Chairperson: F. Gage, *Salk Institute for Biological Studies, La Jolla, California*

Plasticity within and between Cells
Chairperson: D. Johnston, *University of Texas, Austin*

Structural Plasticity and Memory
Chairperson: Y. Goda, *University College London, United Kingdom*

Local and Distributed Synaptic Processing
Chairperson: M. Ehlers, *Duke University Medical Center, Durham, North Carolina*

Encoding of Memory in Cortical Networks
Chairperson: E. Moser, *Norwegian University of Science and Technology, Trondheim, Norway*

Learning, Memory, and Transcription Factors
Chairperson: C. Alberini, *Mount Sinai School of Medicine, New York*

Complexities of Simple Systems
Chairperson: D. Baxter, *University of Texas-Houston Medical School*

Reconsolidation and Extinction
Chairperson: Y. Dudai, *Weizmann Institute of Science, Rehovot, Israel*

M. Collado, J. Pita-Almenar

S. Rose, K. Lukowiak

The Ubiquitin Family

April 27–May 1 270 participants

ARRANGED BY **Joan Conaway,** Stowers Institute for Medical Research
Ray DeShaies, California Institute of Technology
Peter Howley, Harvard Medical School

This was the second Ubiquitin Family meeting following its successful inauguration in 2003. The meeting focused on ubiquitin and the structurally related "ubiquitin-like" proteins, and their regulation in cellular processes. The meeting focused on the central role of ubiquitin and ubiquitin-like protein modifications in protein regulation and turnover. Critical questions in the field relate to molecular basis of specificity with regards to the recognition of the various ubiquitin family members and to the molecular mechanisms that underlie substrate specificity for the ubiquitin and ubiquitin-like protein enzymatic pathways. Research in the area of ubiquitin and related molecules has continued to grow remarkably in the past few years, with an appreciation of how these molecules affect normal and abnormal physiologic processes.

P. Howley

The meeting this year attracted more than 270 scientists who discussed the role of ubiquitin and ubiquitin-like molecules in a variety of key cellular processes, including chromatin structure and transcription, cell cycle, signaling, apoptosis, endocytosis, membrane trafficking, and pathology. The reported experimental systems ranged from yeast to humans, whereas 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 has become a unifying forum that is helping us understand the key roles that ubiquitin and the related ubiquitin-like molecules have in cellular physiology and disease.

Major sponsorship for this meeting was provided by the National Institutes of Health. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

J. Burton, M. Solomon

PROGRAM

Cell Signaling Pathways
Chairpersons: S. Reed, *Scripps Research Institute, La Jolla, California;* J.-M. Peters, *Research Institute of Molecular Pathology, Vienna, Austria*

Enzymes of Ubiquitin Family Protein Systems
Chairpersons: B. Schulman, *St. Jude Children's Research Hospital, Memphis, Tennessee;* C. Pickart, *Johns Hopkins University, Baltimore, Maryland*

Endocytosis and Membrane Trafficking; Proteasome
Chairpersons: L. Hicke, *Northwestern University, Evanston, Illinois;* P. DiFiore, *FIRC Institute of Molecular Oncology, Milan, Italy*

Protein Quality Control
Chairpersons: M. Hochstrasser, *Yale University, New Haven, Connecticut;* T. Sommer, *Max-Delbrück Center for Molecular Medicine, Berlin, Germany*

Regulation and Dysregulation in the Brain
Chairpersons: J. Kaplan, *Harvard Medical School, Boston, Massachusetts;* T. Dawson, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Chromatin Structure, Gene Regulation, and DNA Repair
Chairpersons: S. Berger, *Wistar Institute, Philadelphia, Pennsylvania;* W. Tansey, *Cold Spring Harbor Laboratory*

Cell Cycle Regulation and Cancer
Chairpersons: J.W. Harper, *Harvard Medical School, Boston, Massachusetts;* W. Kaelin, *Dana-Farber Cancer Institute, Boston, Massachusetts*

New Technologies and Therapeutic Strategies
Chairpersons: M. Tyers, *Samuel Lunenfeld Research Institute, Toronto, Canada;* H. Ploegh, *Harvard Medical School, Boston, Massachusetts*

A. Kobayashi, Q. Ma

K. Ross, S. Plafker

Telomeres and Telomerase

May 4–8 286 participants

ARRANGED BY **Titia de Lange,** The Rockefeller University
Joachim Linger, Swiss Institute for Experimental Cancer Research
Vicki Lundblad, Salk Institute for Biological Studies

The conference consisted of eight sessions of talks and three poster sessions. As in 1999, 2001, and 2003, the format was to invite two chairs per session, who were a mix of established scientists in the field and younger scientists who had already made their mark by publishing as independent investigators. Many session chairs gave a scientific (12 min) presentation. The rest of the presentations (also 12 min) were chosen from submitted abstracts, allowing as many presentations as possible. These presentations were primarily by graduate students and postdoctoral fellows. Attendance totalled 286, a high fraction of whom presented the 118 posters and 73 talks.

V. Lundblad, T. de Lange

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 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 2007.

Major sponsorship for this meeting was provided by the National Institute on Aging, a branch of the National Institutes of Health; and the National Science Foundation. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

S. Ahmed, E. Blackburn

PROGRAM

Telomerase Structure and Biochemistry
Chairpersons: K. Collins, *University of California, Berkeley;* N. Lue, *Weill Medical College of Cornell University, New York*

Telomere Length Regulation I
Chairpersons: E. Blackburn, *University of California, San Francisco;* V. Geli, *CNRS, Marseille, France*

Telomere Length Regulation II
Chairpersons: J. Lingner, *Swiss Institute for Experimental Cancer Research, Epalinges;* D. Shippen, *Texas A&M University, College Station*

End Protection and Resection
Chairpersons: V. Lundblad, *Salk Institute for Biological Studies, LaJolla, California;* R. Wellinger, *University of Sherbrooke, Canada*

Consequences of Telomere Dysfunction
Chairpersons: T. de Lange, *The Rockefeller University;* M. Longhese, *Università di Milano-Bicocca, Italy*

Telomeres and Recombination
Chairpersons: R. Reddel, *Children's Medical Research Institute, Westmead, Australia;* E. Louis, *University of Leicester, United Kingdom*

Structural Studies of Telomeres and Telomere Proteins
Chairpersons: V. Zakian, *Princeton University, New Jersey;* P. Lansdorp, *British Columbia Cancer Research Center, Vancouver, Canada*

Senescence, Apoptosis, and Cancer
Chairpersons: C. Greider, *Johns Hopkins University School of Medicine, Baltimore, Maryland;* W. Wright, *University of Texas Southwestern Medical Center, Dallas*

C. Greider, S. Smith

L. Rudolph, S. Artandi

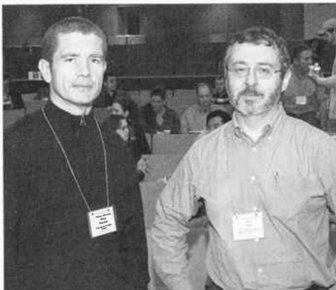
Workshop on Chicken Genomics and Development

May 8–11 121 participants

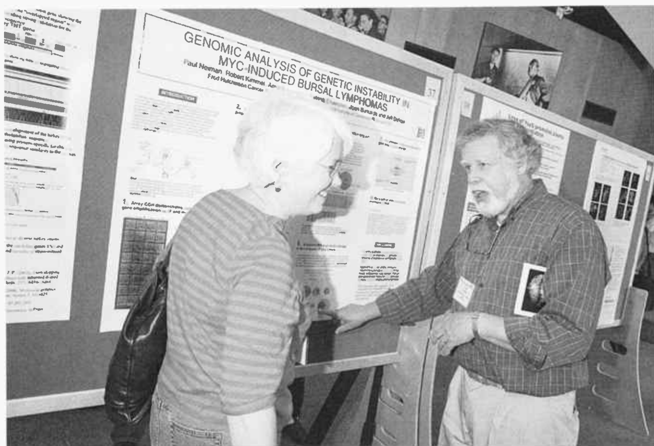
ARRANGED BY **Dave Burt,** Roslin Institute
Olivier Pourquie, Stowers Institute for Medical Research

This meeting marked the third annual gathering of some of the premier researchers in the field from around the world. The chicken is now clearly recognized as a model genome and is of tremendous value in evolutionary comparisons.

The meeting was attended by 121 researchers with 76 abstracts presented, representing submissions from scientists in 16 countries. The workshop began with a keynote presentation by Lincoln Stein, who described the most recent venture into the use of computational tools such as the "Genetic Model Organism Database" (GMOD). Scientific presentations covered genetic resources (such as SNPs, haplotype analysis, and QTL identification), expression profiling, and sequence to function. Additional discussions centered on gynandromorphs, endogenous viral elements, and transgenesis, serving to emphasize some of the novel approaches now in progress. Presentations during the final developmental biology session highlighted efforts on developmental mutants and signaling pathways. A wrap-up of the session included an exploration of "What Now" and "What Next," with plans to continue these meetings at Cold Spring Harbor. The results of this meeting resulted in a clear map as to what precisely should happen next, beginning with a plan to hold another Cold Spring Harbor meeting in 2006.



O. Pourquie, D. Burt



K. Beemon, P. Neiman

Major sponsorship for this meeting was provided by ARK-Genomics, Applied Biosystems, Affymetrix, Cobb Vantress Inc., Aviagen, Genesis Faraday, and Developmental Dynamics. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

PROGRAM

Opening Remarks
D. Burt, *Roslin Institute, United Kingdom*

Plenary Session: Model Organism Databases
L. Stein, *Cold Spring Harbor Laboratory*

Analysis of the Chicken Genome and the Chicken Gene Set I
Chairperson: J. Dodgson, *Michigan State University, East Lansing*

Analysis of the Chicken Genome and the Chicken Gene Set II
Chairperson: J. Dodgson, *Michigan State University, East Lansing*

SNPs and QTL Mapping
Chairperson: L. Andersson, *University of Uppsala, Sweden*

Genome Browser Tutorials
Chairperson: J. Burnside, *Delaware Biotechnology Institute, Newark*

Evolutionary Biology
Chairperson: D. Burt, *Roslin Institute, Edinburgh, United Kingdom*

Gene Regulation and Expression
Chairperson: P. Antin, *University of Arizona, Tucson*

The Chick: A Leading Model in Developmental Biology I
Chairperson: O. Pourquié, *Stowers Institute for Medical Research, Kansas City, Missouri*

K. Reed, P. Mariani

M. Miller, R. Taylor

The Biology of Genomes

May 11–15 512 participants

ARRANGED BY **Kelly Frazer,** Perlegen
Thomas Hudson, McGill University
Svante Pääbo, Max-Planck Institute
Richard Wilson, Washington University

This meeting marked the 16th annual gathering of genome scientists in this setting. The past decade or more has seen remarkable progress in the mapping, sequencing, and annotation of the genomes of many "model organisms" and publication of finished and draft sequences of the human, mouse, rat, and dog genomes, in addition to several model organisms and hundreds of bacteria. Just over 500 people from around the world attended the meeting, with more than 300 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

The session topics included areas such as biological insights from comparative genomics, functional genomics, organismal biology, computational genomics, and the use of sequence variations to study populations and mechanisms of disease. There was considerable enthusiasm and discussion regarding current international projects such as the HapMap and Encode. Once again, projection-style, interactive computer demonstrations in Grace Auditorium effectively highlighted the critical new bioinformatics tools being developed for storing, organizing, and analyzing various genomic data sets, including expression, mapping, and sequence data. There was also an ELSI (Ethical, Legal, and Social Implications) panel discussion that focused on issues surrounding (large-scale) medical resequencing.

The major themes of the meeting included the analysis of large-scale variation in genetic variation across the human genome (with considerable interest with regard to new insight in structural variation), annotation of the human genome using multispecies sequence comparisons, and a myriad of large-scale approaches to understand complex biological processes. The Saturday afternoon keynote talks were delivered by Aravinda Chakravarti, and Tom Gingeras (a former research scientist at CSHL.

Major sponsorship for the meeting was provided 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 affiliates, corporate contributors, and foundations also provided core support for this meeting.

A. Caudy, J. Hudson

High-throughput Biology
Chairpersons: E. Green, *NHGRI, National Institutes of Health, Bethesda, Maryland;* B. Andrews, *University of Toronto, Canada*

Computational Genomics
Chairpersons: D. Haussler, *University of California, Santa Cruz;* E. Eichler, *University of Washington, Seattle*

Perturbations of Genome Systems
Chairpersons: C. Austin, *NHGRI, National Institutes of Health, Bethesda, Maryland;* A. Fraser, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Haplotype Variation and Intraspecies Resequencing
Chairpersons: T. Hudson, *McGill University and Genome Quebec Innovation Centre, Montreal, Canada;* E. Mardis, *Washington University, St. Louis, Missouri*

M. Kellis, F. Collins, R. Waterston

ELSI Panel Discussion: Medical Sequencing: Human Genome Sequencing of Phenotyped Individuals for Large Population Studies
Moderator: V. Ota Wang, *National Human Genome Research Institute*
Panelists: M.A. Spence, *University of California, Irvine*; J.R. Botkin, *University of Utah*; M.W. Foster, *University of Oklahoma*

SNPs and Their Relationship to Biology
Chairpersons: V. Cheung, *Children's Hospital, Philadelphia, Pennsylvania;* R. Gibbs, *Baylor College of Medicine, Houston, Texas*

Non-Human Species Including Comparative Genomics of Distantly Related Species
Chairpersons: R. Hardison, *Pennsylvania State University, University Park;* K. Lindblad-Toh, *Broad Institute of MIT and Harvard, Cambridge, Massachusetts*

Keynote Speakers
T.R. Gingeras, *Affymetrix;* A. Chakravarti, *Johns Hopkins University School of Medicine*

Evolutionary Biology Including Comparative Genomics of Closely Related Species
Chairpersons: C. Langley, *University of California, Davis;* T. Mikkelsen, *Broad Institute of MIT and Harvard, Cambridge, Massachusetts*

A. Alejandro-Osorio

H. Sussman, M. Smit, X. Tordoir, A. Clop

Protein Phosphorylation and Cell Signaling

May 18–22 215 participants

ARRANGED BY **Nicholas Tonks,** Cold Spring Harbor Laboratory
Sara Courtneidge, Van Andel Research Institute
Ben Neel, Beth Israel Deaconess Medical Center

This sixth meeting brought together 215 scientists from the United States, Europe, the Far East, and South Pacific. 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 Martine Roussel and Craig Thompson.

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. Therefore, the program for this meeting addressed the role of protein phosphorylation in the regulation of signal transduction under normal and pathophysiological conditions.

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.

Major sponsorship for this meeting was provided by the National Cancer Institute, a branch of the National Institutes of Health. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

N. Tonks, S. Courtneidge, B. Neel

Keynote Speakers
M. Roussel, *St. Jude Children's Research Hospital, Memphis, Tennessee*
C. Thompson, *University of Pennsylvania*

Receptor-proximal Signaling
Chairperson: D. Cantrell, *University of Dundee, United Kingdom*

Physiology and Disease
Chairperson: J. Dixon, *University of California, San Diego*

Downstream Signaling
Chairperson: J. den Hertog, *Hubrecht Laboratory, Utrecht, The Netherlands*

Cancer
Chairperson: K. Gould, *Vanderbilt University, Nashville, Tennessee*

Metabolic and Stress Signaling
Chairperson: R. Abraham, *The Burnham Institute, La Jolla, California*

Model Systems
Chairperson: W. Muller, *McGill University, Montréal, Canada*

Trafficking and the Cytoskeleton
Chairperson: D. Bar-Sagi, *Stony Brook University, New York*

R. Yacobi, E. Wegener

T. Camiulli, L. Krilov

S. Yang, G. van Nieuw Amerongen

Retroviruses

May 24–29 430 participants

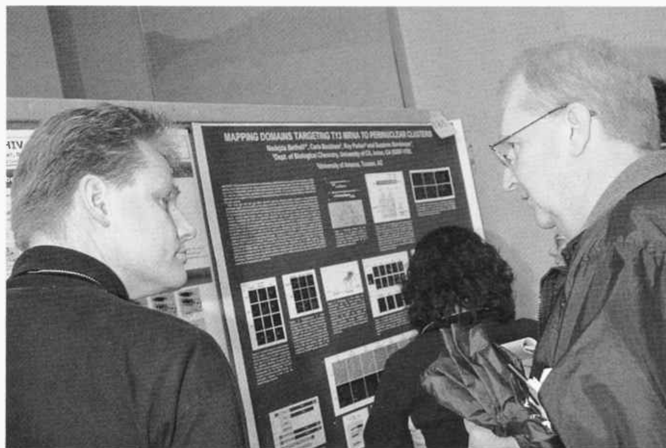
ARRANGED BY **Paul Bieniasz,** Aaron Diamond AIDS Research Center
James Cunningham, Harvard Medical School

The 30th annual Retrovirus meeting was organized into three keynote addresses, ten sessions of short talks on specific topics, and three poster sessions.

Three distinguished scientists delivered keynote addresses on consecutive evenings. On Wednesday, Dr. Wesley Sundquist gave a scholarly summary of recent advances in understanding retrovial capsid structure and function. In particular, he outlined rules for capsid oligomerization during virion assembly and maturation. On Thursday, Dr. Robert Craigie gave a historical perspective on studies of integrase structure and function, including the role of host factors. On Friday, Dr. Beatrice Hahn discussed her new and exciting field studies on the origin and evolution of HIV from primate lentiviruses in Africa.

Ten sessions were devoted to short talks on specific topics including virus entry, assembly, restriction factors, postentry, integration, APOBEC, accessory genes, pathogenesis, reverse transcription, and RNA synthesis and export. The keynote addresses served, in part, as an introduction to the ensuing oral sessions. Poster sessions were organized by topic such that posters dealing with closely related themes were placed together, the goal being to bring scientists with common interests into proximity. Many presenters liked this organization, but there are also some who still prefer the posters to be arranged alphabetically.

Although the most striking developments this year involved postentry events, significant progress was made toward elucidating a number of aspects of the retroviral replication cycle. Highlights included recent progress in understanding the mechanism by which TRIM5α inhibits retroviral infection, how the lentiviral Vif protein counteracts the antiviral effects of the cellular enzyme APOBEC3G, and the



J. Cunningham, G. Melikian

identification of host factors that can inhibit postentry events. Provocative findings related to function of retroviral accessory proteins Nef, Vpr, and Bet were also reported. Two new retrovirus receptors were identified and several new experimental approaches to analyze virus entry were presented. In addition, new measurements of the efficacy of antiviral therapy were reported. A major theme was the development of screening protocols to identify host factors that inhibit retroviral infection. It is anticipated that further identification and analysis of host factors that act in both a positive and negative manner to influence retrovirus replication will be a major topic at future meetings.

In other matters, the meeting is increasingly more user-friendly. Strengths are the improved food, banquet, and A-V/computer support. Weaknesses are off-sight housing, proximity to holiday, length of evening sessions, and the small size of the bar. This year, poor weather disrupted many of the outdoor activities. Finally, we very much appreciate the help and guidance of David Stewart, Mark Beavers, and the CSHL staff, who made the meeting easy and enjoyable to organize.

Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations provided core support for this meeting.

A. Rein, L. Parent

G. Towers, M. Yap, K. Bishop

J. Blomberg, S. Hughes

PROGRAM

Entry
Chairpersons: J. Silver, *National Institutes of Health, Bethesda, Maryland;* D. Sanders, *Purdue University, West Lafayette, Indiana*

Restriction Factors
Chairpersons: V. KewalRamani, *National Cancer Institute, Frederick, Maryland;* J. Stoye, *National Institute for Medical Research, London, United Kingdom*

Keynote Speaker: Structure and Functions of the Retroviral Capsid
Wes Sundquist, *University of Utah School of Medicine*

Assembly I
Chairpersons: H. Gottlinger, *Dana-Farber Cancer Institute, Boston, Massachusetts;* J. Lingappa, *University of Washington, Seattle*

Assembly II
Chairpersons: C. Carter, *Stony Brook University, New York;* W. Mothes, *Yale University, New Haven, Connecticut*

Keynote Speaker: Retroviral Integration: An Odyssey from Genetics to Chemistry and Beyond
Robert Craigie, *NIDDK, National Institutes of Health*

Postentry
Chairpersons: A. Fassati, *University College London Medical School, United Kingdom;* G. Kalpana, *Albert Einstein College of Medicine, Bronx, New York*

Integration / Bioinformatics
Chairpersons: A. Engelman, *Dana-Farber Cancer Institute, Boston, Massachusetts;* E. Poeschla, *Mayo Clinic College of Medicine, Rochester, Minnesota*

Keynote Speaker: Natural Reservoirs of HIV-1
Beatrice Hahn, *University of Alabama, Birmingham*

Accessory Genes
Chairpersons: T. Hope, *University of Illinois, Chicago Medical School;* P. Jolicoeur, *Institut de Recherches des Cliniques de Montréal, Canada*

APOBEC
Chairpersons: R. Harris, *University of Minnesota, Minneapolis;* K. Strebel, *NIAID, National Institutes of Health, Bethesda, Maryland*

Reverse Transcription / Pathogenesis
Chairpersons: M. Gotte, *McGill University, Montréal, Canada;* M. Roth, *UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey*

RNA Synthesis, Export and Packaging
Chairpersons: B. Felber, *National Cancer Institute, Frederick, Maryland;* A. Rice, *Baylor College of Medicine, Houston, Texas*

J. Levin, A. Telesnitsky

New York Structural Biology Group

August 10 226 participants

ARRANGED BY **Leemor Joshua-Tor,** Cold Spring Harbor Laboratory
Larry Shapiro, Columbia University College of Physicians & Surgeons
David Stokes, Skirball Institute, New York University

D. Stokes, L. Joshua-Tor, L. Shapiro

The summer meeting of the New York Structural Biology Discussion Group was the seventh in day-long meetings allowing structural biologists from all over the region to meet and discuss their latest results. The meeting was open to structural biologists from different disciplines, including crystallographers, spectroscopists, computational biologists, and biochemists with more than 250 participants from academia and industry from the tristate area. The program featured 12 talks, a poster session and concluded with a beach barbecue, allowing a wonderful opportunity for informal interactions. This meeting complements the bimonthly evening meetings of the group held at The Rockefeller University. No registration was required and participants were encouraged to set up posters.

Major sponsorship for this meeting was provided by Fluidigm, GE Healthcare, Hampton Research, and Merck. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

H. Robinson, L. Berman

L. Joshua-Tor, W. Hendrickson, R. Spencer

PROGRAM

Session I

Chairpersons: L. Shapiro, *Columbia University College of Physicians & Surgeons*; H. Wu, *Weill Medical College of Cornell University*; C. Wang, *Columbia University College of Physicians & Surgeons (Palmer lab)*; J. Truglio, *Stony Brook University (Kisker lab)*; J. Ferrara, *Rigaku/MSC*

Session II

Chairpersons: D. Stokes, *Skirball Institute, New York University Medical School*; D. Patel, *Memorial Sloan-Kettering Cancer Center*; Y. Song, *City College of New York (Gunner lab)*; D. Jain, *The Rockefeller University (Darst lab)*; D. Raleigh, *Stony Brook University*

Poster Session

Session III

Chairpersons: L. Joshua-Tor, *Cold Spring Harbor Laboratory*; Barry Honig, *Columbia University College of Physicians & Surgeons*; J. Williams, *Columbia University College of Physicians & Surgeons (Hendrickson lab)*; C. Lima, *Memorial Sloan-Kettering Cancer Center*; E. Crocker, *Stony Brook University (Smith lab)*; T. Walz, *Harvard Medical School*

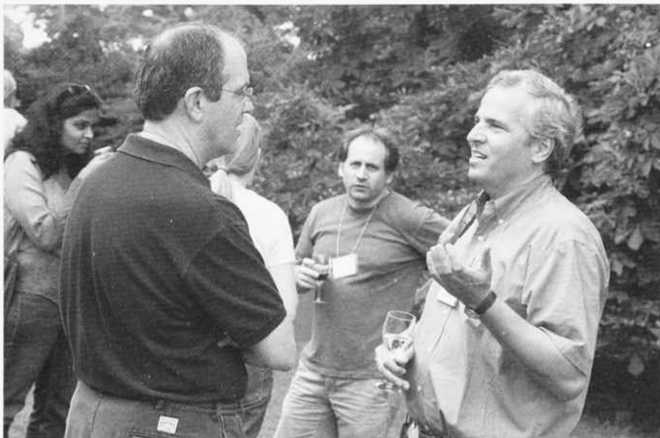
Yeast Cell Biology

August 16–21 245 participants

ARRANGED BY **Kerry Bloom,** University of North Carolina, Chapel Hill
Chris Kaiser, Massachusetts Institute of Technology
Peter Pryciak, University of Massachusetts Medical School

This tenth biannual international meeting was 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*, with additional insights arising from studies using other fungi such as *Ashbya gossypii*, *Candida albicans*, *Pichia pastoris*, and *Schizosaccharomyces pombe*. At this year's conference, the latest research advances were presented in sessions organized around primary cell biological systems. For example, new developments were discussed in the areas of the actin and microtubule cytoskeletons, membrane trafficking, cell cycle and cell division, and signal transduction mechanisms. In addition, functional genomics, proteomics, and bioinformatics were featured prominantly, with remarkable examples of using yeast to study genome-wide changes that accompany evolution. Furthermore, it has become increasingly clear during the last several years that traditional cell biological systems do not operate in isolation. Instead, they are highly coordinated, and the mechanisms of this coordination represent some of the most exciting research areas covered at the meeting, including the organization of membranous organelles by the cytoskeleton, subcellular compartmentalization of cell cycle control proteins, and regulation of cytoskeletal organization by signal transduction pathways. Complementing these integrative topics were new computational models for complex molecular behavior and new microscopic techniques for probing cell ultrastructure. The result is that the yeast system is approaching an unprecedented degree of sophistication in the comprehensive, system-wide understanding of cellular function.

By virtue of this common interest in one organism, instead of one topic in cell biology, this meeting continues to encourage extensive cross-fertilization of ideas, insights and methodologies, ultimately



R. Rothstein, C. Kaiser

leading to an integrated view of eukaryotic cell structure and function. Yeast remains an important test bed for new technological developments and conceptual advances in biomedical sciences. Presentations at the 2005 meeting made clear that yeast will remain at the forefront of model systems and will advance our understanding of eukaryotic cell biology for many years. In summary, this was an exciting and memorable meeting with more than 240 scientists in attendance, presenting some 185 scientific reports in 101 talks and 84 posters.

Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations provided core support for this meeting.

PROGRAM

Microtubules and SPBs
Chairperson: M. Rose, *Princeton University, New Jersey*

Signaling and Networks
Chairperson: D. Lew, *Duke University, Durham, North Carolina*

Trafficking
Chairperson: T. Graham, *Vanderbilt University, Nashville, Tennessee*

Kinetochores and Chromosomes
Chairperson: R. Rothstein, *Columbia University College of Physicians & Surgeons, New York*

Genomes and Genomics
Chairperson: V. Guacci, *Fox Chase Cancer Center, Philadelphia, Pennsylvania*

Actin and Polarity
Chairperson: L. Pon, *Columbia University, New York*

Organelle Biogenesis
Chairperson: T. Fox, *Cornell University, Ithaca, New York*

Physiology
Chairperson: J. Thorner, *University of California, Berkeley*

Membranes and Morphogenesis
Chairperson: J. Berman, *University of Minnesota, Minneapolis*

Cell Cycle and Checkpoints
Chairperson: O. Cohen-Fix, *NIDDK, National Institutes of Health, Bethesda, Maryland*

P. Pryciak, J. Thorner

C. Potenski, M. Kadner

M. Gardner, K. Bloom

Eukaroytic mRNA Processing

August 24–28 405 participants

ARRANGED BY **Elisa Izaurralde,** EMBL Heidelberg
Timothy Nilsen, Case Western Reserve University, Cleveland
Donald Rio, University of California, Berkeley

This fifth meeting focused on recent developments in mRNA metabolism. As in the past, there was a heavy emphasis on regulated and alternative pre-mRNA splicing as well as splicing mechanisms. Additional areas included mRNA quality control and decay, mRNA trafficking, and 3′-end formation. Because of increasing interest and progress, sessions on systems approaches and coupling with other processes (e.g., transcription) were added. Although RNA interference has been covered at this meeting in the past, it was dropped as a session this year because of the new Cold Spring Harbor meeting on RNAi and microRNA function.

In terms of splicing regulation, many specific examples were discussed. Although there has been significant progress in deciphering detailed regulatory mechanisms, there were few unifying themes. Each case appears to be unique, and multiple nonoverlapping factors, both positive and negative, have been identified. In addition, array technology has revealed remarkably complex patterns of tissue-specific and developmental regulation of alternative splicing.

With regard to splicing mechanism, several talks illuminated new proofreading mechanisms that function at both the 5′ and 3′ splice sites. Additionally, surprising results indicate that different introns show differential dependence on constitutive splicing factors. The majority of presentations on 3′-end formation focused on new links between polyadenylation and both transcription and splicing. In addition, identification of the nuclease responsible for 3′-end cleavage was reported.

The RNA trafficking session served to emphasize the complexity of nucleus-cytoplasmic communication. Particularly exciting were presentations describing the interrelationship between processing bodies and stress bodies. Furthermore, the role of helicases in transport is finally being illuminated.

In terms of surveillance and decay, significant progress has been made in the biochemical analysis of the exon junction complex. Nevertheless, it remains unclear how this complex triggers nonsense-mediated decay in higher eukaryotes. Further complications were introduced when NMD was analyzed

T. Nilsen, E. Izaurralde, D. Rio

A. Stevenson, J.-A. Wise

in lower eukaryotes. Even though these organisms have the components of the exon junction complex, these components are not required for surveillance. The nuclease(s) responsible for initiating this type of decay is still unknown.

Finally, the interplay of the cell's environment with the mRNA processing machinery was extensively discussed. It now seems quite clear that signal transduction pathways have dramatic effects on alternative splicing, 3′-end formation, and decay. The mechanisms by which these pathways intersect and effect mRNA maturation remain largely obscure.

Major sponsorship for this meeting was provided by the National Cancer Institute and the National Institute of Child Health and Human Development, branches of the National Institutes of Health; and the National Science Foundation. In addition, the Laboratory would like to thank the RNA Society for its support of this meeting. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

PROGRAM

Systems-Level Approaches to Regulation of mRNA Processing
Chairperson: M. Ares, *University of California, Santa Cruz*

Alternative and Regulated Splicing I
Chairperson: D. Black, *Howard Hughes Medical Institute, University of California, Los Angeles*

RNA Trafficking and Localization
Chairperson: U. Kutay, *Swiss Federal Institute of Technology, Zürich*

Mechanism and Regulation of 3′-end Formation
Chairperson: J. Manley, *Columbia University, New York*

Spliceosome: Mechanism Assembly and Factors
Chairperson: C. Guthrie, *University of California, San Francisco*

Mechanisms of mRNA Decay and Surveillance
Chairperson: A.-B. Shyu, *University of Texas-Houston Medical School*

Alternative and Regulated Splicing II
Chairperson: A. Krainer, *Cold Spring Harbor Laboratory*

Coupling and Networks in Pre-mRNA Processing
Chairperson: M. Rosbash, *Howard Hughes Medical Institute, Brandeis University, Waltham, Massachusetts*

G. Dreyfuss, J. Manley, D. Spector

K. Frato, K. Thickman, K. Xie

Mechanisms of Eukaryotic Transcription

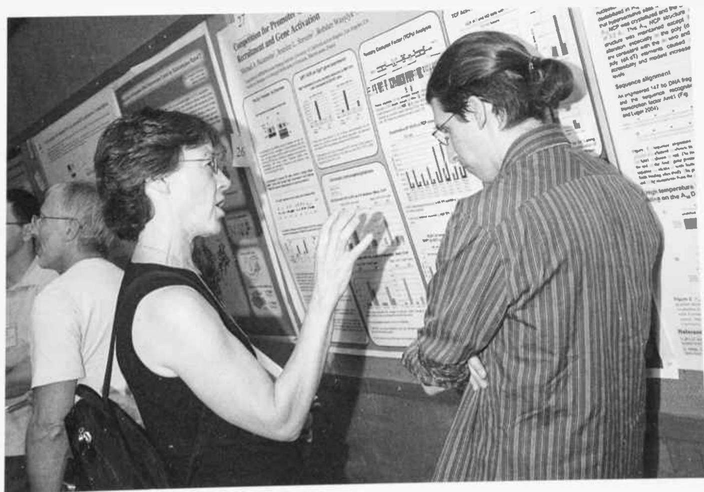
August 31–September 4 453 participants

ARRANGED BY

Joan Conaway, Stowers Institute for Medical Research
Barbara Graves, University of Utah
Jerry Workman, Stowers Institute for Medical Research

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. This meeting appropriately covered all aspects of transcription and brought together a diverse group of scientists. The meeting consisted of eight plenary sessions and three poster sessions. One session focused on the most recent structures of RNA polymerases and their associated transcription factors and on how our understanding of transcription mechanisms is being illuminated by these structures. Two sessions, entitled Histone Modification and Chromatin Remodeling, covered the role of chromatin in transcription control. Additional sessions covered functions of DNA-binding transcriptional regulatory proteins and coregulatory complexes, control of elongation and termination, and signaling pathways that regulate transcription. A final session discussed regulatory networks and mechanisms and included genomic and proteomic approaches.

Major sponsorship for this meeting was provided by the National Cancer Institute and the National Institute of Child Health and Human Development, branches of the National Institutes of Health; and the National Science Foundation. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.



B. Graves, M. Balamotis

PROGRAM

Transcription Complexes
Chairperson: J. Conaway, *Stowers Institute for Medical Research, Kansas City, Missouri*

Signaling
Chairperson: A. Berk, *University of California, Los Angeles*

Structure and Mechanism
Chairperson: R. Tjian, *University of California, Berkeley*

Elongation and Termination
Chairperson: P. Farnham, *University of California, Davis*

Regulatory Networks
Chairperson: R. Treisman, *Cancer Research UK–London Research Institute*

Chromatin Remodeling
Chairperson: S. Mango, *University of Utah, Salt Lake City*

Activators
Chairperson: T. Archer, *NIEHS, National Institutes of Health, Research Triangle Park, North Carolina*

Histone Modifications
Chairperson: G. Almouzni, *Institut Curie, Paris, France*

J. Conaway, H. Handa

A. Eceleston, S. Berger

M. Timmers, I. Davidson

Eukaryotic DNA Replication

September 7–11 331 participants

ARRANGED BY **Stephen Bell,** HHMI/Massachusetts Institute of Technology
Joachim Li, University of California, San Francisco
Terry Orr-Weaver, Whitehead Institute/Massachusetts Institute of Technology

This was the ninth biannual meeting on eukaryotic DNA replication held at Cold Spring Harbor. Important progress has been made in our understanding of eukaryotic DNA replication, and this meeting is crucial in bringing together an international array of researchers investigating all aspects of eukaryotic DNA replication. The most recent advances in the field were presented together with new approaches for analyzing DNA replication, making this meeting the most important in the field. A large number of investigators participated in the ten scientific sessions, with nearly 250 platform and poster presentations. The platform and poster sessions were marked by spirited and enthusiastic exchanges of new results. In the scientific sessions many audience members participated in the question and answer sessions, and the poster sessions were well-attended.

The meeting illustrated the rapid advances in our understanding of the function of replication proteins in initiation and elongation of DNA replication, the surveillance mechanisms monitoring the accuracy of replication, a global genomic view of replication, and the coordination between DNA replication and development.

Major sponsorship for this meeting was provided 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 affiliates, corporate contributors, and foundations also provided core support for this meeting.

B. Calvi, T. Orr-Weaver

PROGRAM

Assembly of Replication Initiation Machines
Chairperson: A.-K. Bielinsky, *University of Minnesota, Minneapolis*

Replication Timing and Origin Activation
Chairperson: B. Brewer, *University of Washington, Seattle*

Genomic Analysis of DNA Replication and Checkpoint Control
Chairperson: K. Shirahige, *Tokyo Institute of Technology, Japan*

Damage Responses and Checkpoint Control
Chairperson: J. Diffley, *Cancer Research UK, South Mimms*

Replication and the Cell Cycle
Chairperson: J. Walter, *Harvard Medical School, Boston, Massachusetts*

Replication Fork Proteins
Chairperson: M. O'Donnell, *Howard Hughes Medical Institute, The Rockefeller University, New York*

Control of Replication during Development
Chairperson: M. Botchan, *University of California, Berkeley*

Origin Selection
Chairperson: D. Gilbert, *SUNY Upstate Medical University, Syracuse*

J. Blow, M. Leffak

S. Waga, M. Weinreich

S. Aves, Y. Kawasaki, K. Moore

Microbial Pathogenesis and Host Response

September 14–18 265 participants

ARRANGED BY **Paula Sundstrom,** Dartmouth Medical School
James Slauch, University of Illinois, Urbana/Champaign
Theresa Koehler, University of Texas, Houston Medical School

Microbial pathogens have coevolved with humans and developed sophisticated mechanisms and to interact with and manipulate their hosts. Thus, despite advances in modern health care, microbial pathogens continue to be major causes of human disease and mortality. The isolation of organisms resistant to all known antimicrobials, as well as the emergence of apparently new pathogens and an increased awareness of the potential use of microbial pathogens as agents of bioterrorism, emphasizes the need for increased understanding of pathogenic mechanisms with the goal of developing new therapies and other preventive strategies.

T. Koehler, P. Sundstrom

Elucidating aspects of microbial pathogenesis requires an interdisciplinary approach, integrating the fields of microbiology, eukaryotic cell biology, and immunology. The meeting attracted a diverse group of international scientists who approach the study of bacterial and fungal pathogens from a broad range of perspectives. The continued participation of a large number of young scientists is particularly noteworthy. The program included a keynote address by Aaron P. Mitchell, Professor of Microbiology at Columbia University. Trained in classical yeast genetics, he is now a leader in the field of fungal pathogenesis and genomics.

The oral sessions included mixtures of established investigators presenting their recent results as well as graduate students and postdoctoral fellows presenting their new findings. Sessions focused on how

K. McIver, T. Kinkel, A. Almengor

certain pathogens establish a niche and grow inside eukaryotic cells, how pathogens alter their surface properties to interact with the host, how the host immune response deals with certain pathogens, and how bacterial toxins are secreted and carry out their biological function. The session on novel vaccines and therapeutic strategies also included a general discussion of the challenges of developing antimicrobials in the modern pharmaceutical industry. The remaining sessions focused on virulence gene regulation in response to the host environment and novel animal models to study host-pathogen interactions. 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 discourses 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 rapidly takes its place. Hence, there will be a continual need for the free, interactive exchange of ideas like that stimulated by this meeting.

Major sponsorship for this meeting was provided by 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 affiliates, corporate contributors, and foundations provided core support for this meeting.

PROGRAM

Intracellular Biology
Chairperson: D. Holden, *Imperial College, London, United Kingdom*

Microbial Surfaces
Chairperson: W. Goldman, *Washington University School of Medicine, St. Louis, Missouri*

Host Responses
Chairperson: S. Levitz, *Boston University, Massachusetts*

Toxin/Effector Delivery and Function
Chairperson: P. Schlievert, *University of Minnesota Medical School, Minneapolis*

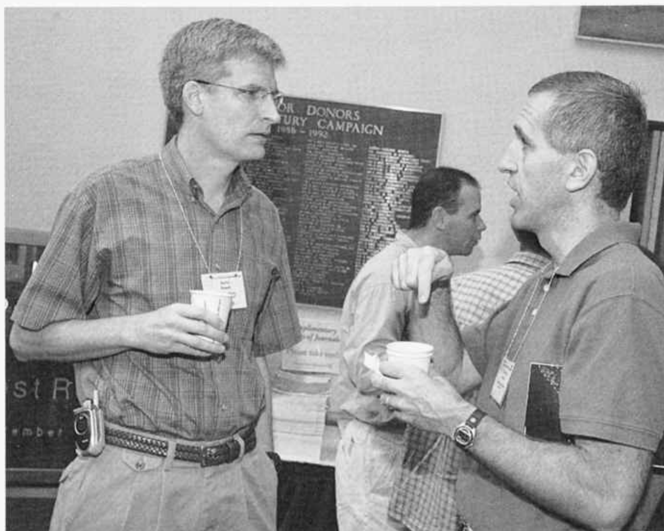
Vaccines
Chairperson: D. Higgins, *Harvard Medical School, Boston, Massachusetts*

Therapeutics
Chairperson: S. Projan, *Wyeth-Ayerst Research, Pearl River, New Jersey*

Gene Regulation
Chairperson: A. Camilli, *Tufts University, Boston, Massachusetts*

Keynote Speaker: The Landscape of Gene Function in *C. albicans*
Chairperson: A. Mitchell, *Institute for Cancer Research, Columbia University, New York*

Host/Microbe Interactions
Chairperson: V. Miller, *Washington University School of Medicine, St. Louis, Missouri*



J. Slauch, T. Hawn

M. Valvano, S. Goldman

Programmed Cell Death

September 21–25 329 participants

ARRANGED BY **Hermann Steller,** HHMI/Massachusetts Institute of Technology
Jurg Tschopp, University of Lausanne, Switzerland
Jungying Yuan, Harvard Medical School

The opening keynote address formed part of the opening session on Mechanisms and Functions of the Bcl-2 Family and was delivered by Craig Thompson on Programmed Cell Death: The New Frontiers. Thompson's laboratory has pioneered the study of the Bcl-2 family of oncogenes, or cancer-causing genes, and their role in regulating cell survival. On the basis of this research, future treatments could be designed to block the ability of cancer cells to survive and thus limit tumor size and prevent the cancer from spreading. The opening session was followed by seven oral sessions and two poster sessions. In all, the meeting featured 234 presentations and was attended by participants from more than 20 countries.

Major sponsorship for this meeting was provided by the National Institute on Aging, the National Cancer Institute, and the National Institute of Child health and Human Development, branches of the National Institutes of Health; and the National Science Foundation. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

H. Stellar, M. Jaatella

J. Yuan, J. Tschopp

PROGRAM

Keynote Address: Programmed Cell Death: The New Frontiers
C. Thompson, *Abramson Family Cancer Research Institute, University of Pennsylvania*

Mechanisms and Functions of BCL-2 Family
Chairpersons: A. Strasser, *Walter & Eliza Hall Institute of Medical Research, Parkville, Australia;* Y. Tsujimoto, *Osaka University Medical Center, Japan*

Regulation of Apoptosis in Invertebrates
Chairpersons: K. White, *Massachusetts General Hospital, Harvard Medical School, Charlestown;* D. Xue, *University of Colorado, Boulder*

Apoptotic Signaling
Chairpersons: J.M. Hardwick, *Johns Hopkins University, Baltimore, Maryland;* S. Nagata, *Osaka University Medical School, Japan*

Apoptosis in Cancers
Chairpersons: S. Lowe, *Cold Spring Harbor Laboratory;* E. White, *Rutgers University, Piscataway, New Jersey*

Neuronal Cell Death
Chairpersons: V. Dawson, *Johns Hopkins University of School of Medicine, Baltimore, Maryland;* P. Nicotera, *University of Leicester, United Kingdom*

Regulation of Autophagy and Alternative Cell Death Pathway
Chairpersons: M. Jäätelä, *Danish Cancer Society, Copenhagen;* B. Levine, *University of Texas Southwestern Medical Center, Dallas*

Mechanism and Functions of Caspases
Chairpersons: T. Mak, *University Health Network, Toronto, Canada;* Y. Shi, *Princeton University, New Jersey*

Ubiquitination in Apoptosis
Chairpersons: Y. Lazebnik, *Cold Spring Harbor Laboratory;* X. Wang, *University of Texas Southwestern Medical Center, Dallas*

Summary
Hermann Steller, *The Rockefeller University, New York*

K. Nakanishi, N. Morishima

P. Skoutzos, N. Mulherkar

RNAi

September 28–October 2 257 participants

ARRANGED BY **Greg Hannon,** Cold Spring Harbor Laboratory/HHMI
Phillip Zamore, University of Massachusetts Medical School

This was the first meeting on RNA interference (RNAi) held at Cold Spring Harbor. Although the field is young, studies of RNAi and the related microRNA pathway are advancing rapidly. There were 9 scientific sessions and 53 platform and 71 poster presentations. Richard Jorgensen, the pioneering plant biologist who in 1990 first reported the RNA silencing phenomenon, "cosuppression," delivered the keynote address. Interest in the mechanisms and regulation of RNAi in eukaryotic cells continues to increase, and we hope that this meeting will grow to be the key meeting for the field, fostering the exchange of new ideas and experimental approaches.

Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations provided core support for this meeting.

P. Zamore, T. Holen

C. Rosenau, S. Engle

PROGRAM

Keynote Speaker
R. Jorgensen, *University of Arizona*

Small RNA Biogenesis and Function
Chairperson: T. Nilsen, *Case Western Reserve University, Cleveland, Ohio*

Nuclear Roles for Small RNAs
Chairperson: E. Sontheimer, *Northwestern University, Evanston, Illinois*

Biological Functions of miRNAs
Chairperson: P. Zamore, *University of Massachusetts Medical School, Worcester*

Experimental Uses of RNA Silencing I
Chairperson: S. Hammond, *University of North Carolina, Chapel Hill*

miRNA Expression and Targets
Chairperson: R. Plasterk, *Hubrecht Laboratory, Utrecht, The Netherlands*

Experimental Uses of RNA Silencing II
Chairperson: G. Hannon, *Cold Spring Harbor Laboratory*

Small RNAs, Small Details
Chairperson: L. Joshua-Tor, *Cold Spring Harbor Laboratory*

M. Ghildiyal, J. Broderick

C. Dean, D. Chalker

Neurobiology of *Drosophila*

October 5–9 429 participants

ARRANGED BY **Leslie Griffith,** Brandeis University
Davie Van Vactor, Harvard Medical School

The goal of this 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 by session chairs from the abstracts submitted. 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 cross-fostering of ideas that was valuable to everyone in the field.

Major sponsorship for this meeting was provided 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 affiliates, corporate contributors, and foundations also provided core support for this meeting.

L. Griffith, S. Crews, C. Klämbt, D. Van Vector

PROGRAM

Behavior
Chairperson: R. Davis, *Baylor College of Medicine, Houston, Texas*

Sensory Systems
Chairperson: L. Vosshall, *The Rockefeller University, New York*

Peter Kolodziej Memorial Lecture: Neuronal Function
Chairperson: B. Stewart, *University of Toronto, Canada*

Cell Biology and Pathology
Chairperson: N. Bonini, *University of Pennsylvania, Philadelphia*

Synapse Formation
Chairperson: V. Budnik, *University of Massachusetts, Amherst*

Glial Biology
Chairperson: V. Auld, *University of British Columbia, Vancouver, Canada*

Elkins Memorial Lecture
Chairperson: Mathias Wernet, *Stanford University*

Process Formation
Chairperson: A. Kolodkin, *Johns Hopkins University School of Medicine, Baltimore. Maryland*

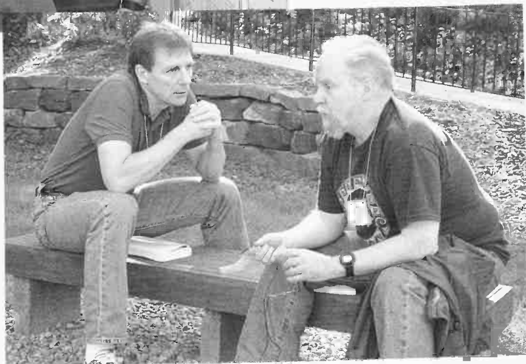
Cell Fate Specification
Chairperson: S. Thor, *Linkoping University, Sweden*

Visual System Development
Chairperson: T. Wolff, *Washington University School of Medicine, St. Louis, Missouri*

Emerging Technologies
Chairpersons: L. Griffith, *Brandeis University, Waltham, Massachusetts;* D. Van Vactor, *Harvard Medical School, Boston, Massachusetts*

I. Meinertzhagen, A. Rodal, N. Giagtzogiou

E. Glater, J. Ashley



R. Davis, J. Truman

Genome Informatics

October 28–November 1 228 participants

ARRANGED BY **Tim Hubbard,** Wellcome Trust Sanger Institute
Suzanna Lewis, University of California, Berkeley
Lincoln Stein, Cold Spring Harbor Laboratory

The fifth Cold Spring Harbor Laboratory–Wellcome Trust conference gave participants the opportunity to enjoy Halloween festivities at Cold Spring Harbor. This conference series alternates between Cold Spring Harbor and the Wellcome Trust Genome Campus south of Cambridge, United Kingdom and follows a format similar to that of traditional Cold Spring Harbor meetings, in that the majority of oral presentations are 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. In addition to the organizers, discussion leaders included Zhirong Bao, Christopher Burge, Thomas Down, Anne Ferguson-Smith, Jennifer Harrow, Robert Martienssen, Webb Miller, Francis Ouellette, Roded Sharan, Lisa Stubbs, Jason Swedlow, and Paul Thomas. The keynote talk was given by David Haussler. In all, 228 participants attended, with more than 25% of delegates coming from outside North America, and the meeting hosted 169 scientific presentations in talks and posters.

Major sponsorship for this meeting was provided 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 affiliates, corporate contributors, and foundations also provided core support for this meeting.

J. Loveland, P. Good

C. Lawrence, R. Voelker

High-throughput Genomics
Chairpersons: J. Harrow, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* B.F.F. Ouellette, *University of British Columbia, Vancouver, Canada*

Pathways and Proteomics I
Chairpersons: P. Thomas, *Applied Biosystems, Foster City, California;* R. Sharan, *Tel Aviv University, Israel*

Pathways and Proteomics II
Chairpersons: P. Thomas, *Applied Biosystems, Foster City, California;* R. Sharan, *Tel Aviv University, Israel*

Keynote Address
D. Haussler, *Howard Hughes Medical Institute, University of California, Santa Cruz*

Epigenomics
Chairpersons: A. Ferguson-Smith, *University of Cambridge, United Kingdom;* R. Martienssen, *Cold Spring Harbor Laboratory*

Birds of a Feather

Images, Atlases, and Phenotypes
Chairpersons: J. Swedlow, *University of Dundee, United Kingdom*; Z. Bao, *University of Washington, Seattle*

Genetic Regulation
Chairpersons: T. Down, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* C. Burge, *Massachusetts Institute of Technology, Cambridge*

Comparative and Evolutionary Genomics I
Chairpersons: W. Miller, *Pennsylvania State University, University Park;* L. Stubbs, *Lawrence Livermore National Laboratory, California*

Comparative and Evolutionary Genomics II
Chairpersons: W. Miller, *Pennsylvania State University, University Park*; L. Stubbs, *Lawrence Livermore National Laboratory, California*

Expect the Unexpected
Chairpersons: T. Hubbard, *Wellcome Trust Sanger Institute*; S. Lewis, *University of California, Berkeley*; L. Stein, *Cold Spring Harbor Laboratory*

L. Krishnan, W. Spooner

M. Ashburner, T. Fiedler

Vector Targeting Strategies for Gene Therapy

November 11–13 94 participants

ARRANGED BY **David T. Curiel,** University of Alabama, Birmingham
Michael Barry, Baylor College
Stephen Russell, Mayo Foundation
Jan Schnitzer, Sidney Kimmel Cancer Center

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 that the vector system recognizes 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 2003, progress has been noted in this field. Especially noteworthy was the progress achieved in the content of viral vector tropism modifications and 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 and providing targeted vector agents for human clinical translation.

Major sponsorship for this meeting was proviced by Biogen Idec, C.G.T. Corporation, Cell Genesys, Inc.; GenVec, Inc.; Louisiana Gene Therapy Research Consortium, Inc; VectorLogics, Inc.; and Wyeth Vaccines Research. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

D. Curiel, S. Hedley

Opening Day
D. Curiel, *University of Alabama, Birmingham*

Keynote Speaker
Chairperson: T. Waldmann, *National Cancer Institute, Bethesda, Maryland*

Adenovirus
Chairperson: M. Barry, *Baylor College of Medicine, Houston, Texas*

AAV
Chairperson: K. Warrington, *University of Florida College of Medicine*

Invited Speaker and Breakout Session
Chairperson: D. Curiel, *University of Alabama, Birmingham*

RNA/Retrovirus
Chairperson: S. Russell, *Mayo Clinic College of Medicine, Rochester, Minnesota*

Cell Vehicle and Nonviral Vector
Chairperson: M. Everts, *University of Alabama, Birmingham*

Imaging and Miscellaneous
Chairperson: M. Yamamoto, *University of Alabama, Birmingham*

D. Brough, M. Trepel

N. Wolfrum, S. Schule

J. Weinstein, M. Nakayama, H. Ugai

Molecular Approaches to Vaccine Design

December 1–4 85 participants

ARRANGED BY **Peter Beverley,** Edward Jenner Institute for Vaccine Research
Emilio Emini, Wyeth Pharmaceuticals
Susan Swain, Trudeau Institute

This fourth winter conference set out to examine whether the revolution in the understanding of molecular and cell biology and in new technologies that has taken place in recent years had begun to have a real impact on how new vaccines are designed. There were sessions on the immunological basis of vaccine immunity; HIV immunity and vaccines; advances in research into malaria, tuberculosis, and respiratory infections; and a final session on new vaccines and underlying immune concepts. It is clear that at the level of the whole immunized animal, there remain many gaps in understanding of factors that determine how different types of protective immunities are generated and maintained in response to different organisms and in different tissue sites. However, several presentations stressed that too vigorous a primary response can lead to terminal differentiation of T cells, resulting in less generation of memory. Improved methods for detection and quantitation of antigen-specific cells are beginning to shed light on how different immunization protocols result in distinct types of immune responses. Genetic analysis of hosts and pathogens suggests that the diversity of response seen in outbred humans may eventually become predictable.

P. Beverley, D. Doolan

More encouragingly at the level of defined cell types and signaling pathways, particularly in antigen-presenting cells, progress is being made in the definition of ligands and downstream pathways that lead to clearly distinct functions. Toll-like receptor ligands, siRNA, and pharmaceutical agents were all shown to be capable of modulating immune responses in predictable and useful ways and should eventually supplant or at least explain the mode of action of empirically defined adjuvants, which currently perform this function.

The meeting also heard of the strikingly successful trials of new rotavirus and papillomavirus vaccines. The rotavirus vaccine, composed of recombinantly engineered human-bovine reassorted rotaviruses, is effective in preventing and mitigating rotaviral disease in infants, yet the mechanism of protection and the immunological correlates of the protection remain unclear. Although this is a striking example of a successful empirical approach to vaccine development, there remain many diseases for which empirical vaccine development has failed. Hopefully, vaccines for these diseases will eventually be successfully developed as fundamental knowledge of the cellular and molecular basis of immune response increases, and as this knowledge is systematically integrated into vaccine design research. Appreciation of the need for such integration was one of the conference's goals, and the goal was, by all accounts of the participants, successfully achieved.

Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations provided core support for this meeting.

PROGRAM

Immunological Basis of Vaccine Immunity I
Chairperson: E. Emini, *Wyeth Pharmaceuticals, Collegeville, Pennsylvania*

Immunological Basis of Vaccine Immunity II
Chairperson: S. Swain, *Trudeau Institute, Saranac Lake, New York*

HIV-1 Immunity and Vaccines
Chairperson: P. Beverley, *Edward Jenner Institute for Vaccine Research, Compton, United Kingdom*

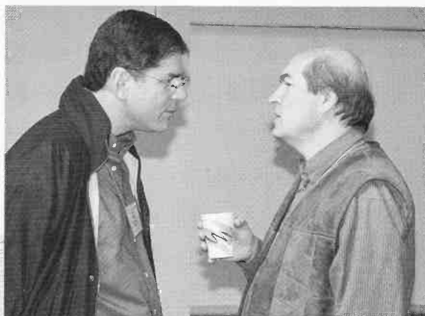
Advances in Malaria and TB Vaccine Research
Chairperson: P. Beverley, *Edward Jenner Institute for Vaccine Research, Compton, United Kingdom*

Respiratory Infections
Chairperson: S. Swain, *Trudeau Institute, Saranac Lake, New York*

CSHL Public Lecture: Bird Flu and the Global Threat of Emerging Respiratory Diseases
Kanta Subbarao, *NIAID, National Institutes of Health, Bethesda, Maryland*

New Vaccines and Underlying Immune Concepts
Chairperson: E. Emini, *Wyeth Pharmaceuticals, Collegeville, Pennsylvania*

J. ter Meulen, A. Hill


D. Galbi, J. Kowalski

M. Page, A. Ojugo

Winter Biotechnology Conference: Rat Genomics and Models

December 8–11 123 participants

ARRANGED BY **Timothy Aitman,** Imperial College, London
Howard Jacob, Medical College of Wisconsin
James Shull, University of Nebraska Medical Center

This fourth winter biotechnology conference followed three successful previous meetings held at the Cold Spring Harbor Laboratory in 1999, 2001, and 2003. Drs. Shull and Aitman joined Dr. Jacob in organizing the conference this year. This series of conferences is the first in the United States to focus exclusively on the rat as a model organism for biological research and directly complements meetings focused on rat genetics/genomics held outside of the United States in the intervening years (i.e., Kyoto Japan in 2002 and Copenhagen Denmark in 2004). The primary goals of this meeting are (1) to promote interactions between biomedical researchers who utilize rat models in the study of physiology, pathophysiology, toxicology, neuroscience, and oncology and (2) to provide an interface between the research community and the various public and commercial resources that exist to support biomedical research in which rat models are utilized.

The meeting was organized into oral presentations by invited speakers as well as speakers selected following evaluation of submitted abstracts, poster sessions, and workshops. Invited speakers included Tim Aitman, Imperial College, London, UK; Artur Rangel-Filho, Medical College of Wisconsin, Milwaukee, WI; Bina Joe, Medical University of Ohio, Toledo, OH; Jonathan Flint, Oxford University, Wellcome Trust Centre, Oxford, UK; Percio Gulko, North Shore Long Island Jewish Research Institute, New York, NY; Colin Bishop, Baylor College of Medicine, Houston, TX; John Critser, University of Missouri, Columbia, MO; Janan Eppig, Jackson Laboratory, Bar Harbor, ME; Howard Jacob, Medical College of Wisconsin, Milwaukee, WI; Norbert Hubner, Max Delbruck Center, Berlin Germany; Janet Young, Fred Hutchinson Cancer Center, Seattle, WA; Linda Moran, Imperial College, London, UK; Nelson, Ruiz-Opazo, Boston University, Boston, MA; Pernilla Stridh-Igo, Karolinska Institute,

H. Jacob, S. Old

Stockholm, Sweden; Eva Redei, Northwestern University, Chicago, IL; Robin Wait, Imperial College, London, UK; Andrea Gore, University Texas, Austin, TX; Michael Waters, NIEHS, Research Triangle Park, NC; Michael Atkinson, GSF-National Research Center for Environment and Health, Neuherberg, Germany. Representatives from the Rat Genome Database presented workshops on the use of this database for accessing data on the rat genome, QTL, and rat strains. This meeting featured 95 presentations and attracted 123 participants from around the world.

Major sponsorship for this meeting was provided by the National Heart, Lung, and Blood Institute, a branch of the National Institutes of Health. Contributions from our corporate benefactors, corporate sponsors, plant corporate associates, corporate affiliates, corporate contributors, and foundations also provided core support for this meeting.

PROGRAM

Positional Cloning
Chairpersons: H. Jacob, *Medical College of Wisconsin, Milwaukee*; T. Serikawa, *Kyoto University, Japan*

Resources I
Chairpersons: T. Aitman, *Imperial College, London, United Kingdom*; J. Ye, *NHLBI, National Institutes of Health, Bethesda, Maryland*

Rat Genome Database Demo and Discussion

Neuroscience
Chairpersons: J. Young, *Fred Hutchinson Cancer Research Center, Seattle, Washington*; E. Cuppen, *Hubrecht Laboratory, Utrecht, The Netherlands*

Proteomics, Expression
Chairpersons: R. Wait, *Imperial College, London, United Kingdom*; N. Hubner, *Max-Delbrück Center for Molecular Medicine, Berlin, Germany*

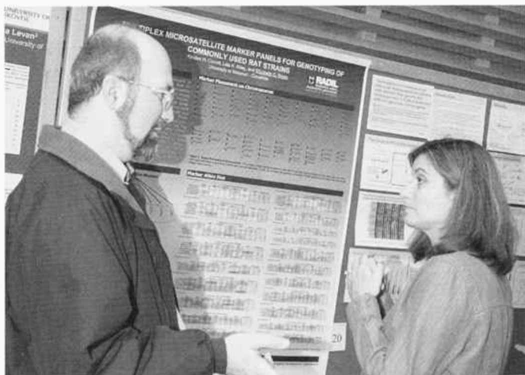
Resources II
Chairpersons: S. Old, *NHLBI, National Institutes of Health, Bethesda, Maryland*; A. Kwitek, *Medical College of Wisconsin, Milwaukee*

Cancer and Induced Neoplasia
Chairpersons: C. Walker, *University of Texas MD Anderson Cancer Center, Smithville*; J. Shull, *University of Nebraska Medical Center, Omaha*

Diabetes
Chairperson: M. Pravenec, *Czech Academy of Sciences, Prague*

Models
Chairpersons: Y. Yagil, *Ben Gurion University, Ashkelon, Israel*; L. Carr, *Indiana University School of Medicine, Indianapolis*

T. Laragione, M. Brenner, T. Aitman



T. Saunders, E. Bryda

CSHL/WELLCOME TRUST CONFERENCES

These conferences were held at the Hinxton Hall Conference Centre, located several miles south of Cambridge in the United Kingdom, which forms part of the Wellcome Trust Genome Campus together with the Sanger Institute and the European Bioinformatics Institute. The conferences are managed jointly by Cold Spring Harbor Laboratory and the Wellcome Trust and follow the Cold Spring Harbor style in that the majority of talks are chosen from openly submitted abstracts. The topics of the joint conference series emphasize genomics and bioinformatics, or topics of particular interest in the United Kingdom and Europe.

Interactome Networks

August 31–September 4 77 participants

ARRANGED BY **Ewan Birney,** European Bioinformatics Institute
Anne-Claude Gavin, Cellzome Inc.
Marc Vidal, Dana-Farber Cancer Institute/Harvard Medical School

The first meeting on Interactome Networks brought together senior and junior investigators and postdoctoral and (post)graduate researchers in a range of disciplines to share existing research and experience. The conference addressed topics including ORFeome and other clone resources and Y2H and other binary assay maps; pull-down mass spectrometry approaches; finishing orthogonal binary assays; assembly/annotation: integration with phenotypic, transcriptome, and localization clustering data; domain-domain networks; and interaction-defective genetics.

PROGRAM

ORFeome
Transcriptional Networks
Binary Maps
Cocomplex Membership Maps

Workshop 1

Interactome Modeling: Functional Integration
Domain Mapping
Disease-related Networks

Workshop 2

Interactome Modeling: Informatics I
Interactome Modeling: Informatics II
Networks and Evolution

Francis Crick Auditorium

Prion Biology

September 7–11 113 participants

ARRANGED BY **Adriano Aguzzi,** University Hospital of Zurich
Bruce Chesebro, Rocky Mountain Laboratories
Mick Tuite, University of Kent
Reed Wickner, National Institutes of Health

This first Prion Biology conference on focused on important advances in prion research in both animals and fungi. Human prions are unique types of infectious agents, whereas fungi prions act as protein-based epigenetic determinants of phenotype. The conference provided a forum to report and discuss the latest discoveries on prions and brought together scientists from a wide range of disciplines in the biological and clinical sciences.

PROGRAM

SESSION 1
M. Tuite, *University of Kent, United Kingdom*

SESSION 2
S. Liebman, *University of Illinois, Chicago*

SESSION 3
A. Aguzzi, *University Hospital Zurich, Switzerland*

SESSION 4
S. Saupe, *University of Bordeaux, France*

SESSION 5
C. Soto, *University of Texas Medical Branch, Galveston*

SESSION 6
B. Caughey, *Rocky Mountain Laboratories, NIAID, Hamilton, Montana*

SESSION 7
R. Wickner, *NIDDK, National Institutes of Health, Bethesda, Maryland*

SESSION 8
B. Chesebro, *Rocky Mountain Laboratories, NIAID, Hamilton, Montana*

Wellcome Trust Conference Center and Hinxton Hall

Pharmacogenomics

September 14–18 120 participants

ARRANGED BY **Steve Leeder,** Children's Mercy Hospital
Debbie Nickerson, University of Washington
Munir Pirmohamed, University of Liverpool
Dick Weinshilboum, Mayo Medical School
C. Roland Wolf, University of Dundee

This third annual conference built on successful conferences held in Hinxton in 2003 and Cold Spring Harbor in the fall of 2004. The meeting again focused on opportunities presented by the growing contribution of emerging genomic information and technologies to interdisciplinary approaches in the study of variable responses of humans to drugs and toxic agents, and how research may benefit the individual. Topics provided an in-depth focus on diverse areas including the biochemistry and physiology of drug action, uptake and metabolism, and how this is affected by genetics; the opportunities for discovery and design of new therapeutic agents; personalizing medicine; understanding and managing adverse drug reactions; the impact of academic and commercial initiatives; and ethical, legal, regulatory, and social consequences of genetics applied to medicine.

PROGRAM

Genome Structure and Variation
Chairpersons: D. Bentley, *(Former) Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* B. Weir, *North Carolina State University, Raleigh*

Molecular Approaches to Phenotype
Chairpersons: J. Nicholson, *Imperial College London, United Kingdom;* T. Nikolskaya, *GeneGo Inc., St. Joseph, Michigan*

Disease Genetics
Chairpersons: C.R. Wolf, *University of Dundee, United Kingdom;* M. Bamshad, *University of Utah, Salt Lake City*

Translation into Clinical Practice
Chairpersons: D. Weatherall, *University of Oxford, United Kingdom;* M. Pirmohamed, *University of Liverpool, United Kingdom*

Societal/Economic Impact and ELSI Issues
Chairpersons: R.M. Weinshilboum, *Mayo Medical School, Rochester, Minnesota;* N.K. Spurr, *GlaxoSmithKline, Stevenage, United Kingdom*

Preclinical Aspects
Chairpersons: S. Leeder, *Children's Mercy Hospital, Kansas City, Missouri;* M. Ingelman-Sundberg, *Karolinska Institutet, Stockholm, Sweden*

Basic Approaches
Chairpersons: D. Balding, *Imperial College London, United Kingdom;* J.P. Ioannidis, *University of Ioannina, Greece*

Grounds of the Wellcome Trust Genome Campus

Functional Genomics of Mammalian Nervous Systems

September 28–October 2 65 participants

ARRANGED BY **Jacqueline Crawley,** National Institute of Mental Health
Seth Grant, Wellcome Trust Sanger Institute
Nathaniel Heintz, HHMI/The Rockefeller University

The theme of this first CSHL/Wellcome Trust conference on Functional Genomics of Mammalian Nervous Systems was to understand the structural and functional complexity of the vertebrate nervous system. The meeting brought together scientists with interests in approaches ranging from molecular biology to behaving animal studies, from single gene to complex sets of genes, and from synapses to networked brain functions. Advances in genomics and proteomics are defining the molecular building blocks that underpin the structural complexity of the brain and its behavioral output. Combining genetic discoveries with anatomical, electrophysiological, and behavioral findings is now making it feasible to integrate this knowledge.

PROGRAM

Behavior
Chairperson: J. Crawley, *NIMH, National Institutes of Health, Bethesda, Maryland*

Welcome and Introduction
Chairperson: Seth Grant, *Wellcome Trust Sanger Institute*

Workshop 1: Databases and Bioinformatics
Chairperson: J.D. Armstrong, *University of Edinburgh*

Brain Gene Expression Mapping and Transcriptome
Chairperson: N. Heintz, *Howard Hughes Medical Institute, The Rockefeller University, New York*

Residential Court

Sanger Institute

Proteomics of the Nervous System
Chairperson: S. Grant, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Workshop 2: Scalable Neuroscience Technology
Chairperson: S.G.N. Grant, *Wellcome Trust Sanger Institute*

Vertebrate Genetic Systems
Chairperson: W. Skarnes, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Human and Primate Studies
Chairperson: J. Noebels, *Baylor College of Medicine, Houston, Texas*

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.

Protein Purification and Characterization

April 6–19

INSTRUCTORS

R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S.-H. Lin, M.D. Anderson Cancer Center/University of Texas, Houston
K. Severinov, Waksman Institute, Rutgers University, Piscataway, New Jersey

ASSISTANTS

K. Adelman, Cornell University, Ithaca, New York
V. Bergendahl, University of Wisconsin, Madison
H. Duong, University of California, Los Angeles
K. Gibson, Massachusetts Institute of Technology, Cambridge
B. Glaser, University of Wisconsin, Madison
Y.-C. Lee, M.D. Anderson Cancer Center/University of Texas, Houston
M. Nie, University of California, Los Angeles
N. Thompson, University of Wisconsin, Madison
C. Winkler, University of California, Los Angeles

This course was for scientists who were not familiar with techniques of protein isolation and characterization. It is a rigorous program that included laboratory work all day and a lecture with a 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. Speakers in the 2005 course included Yuri Lazebnik (Cold Spring Harbor Laboratory), Leemor Joshua-Tor (Cold Spring Harbor Laboratory), Bruce Stillman (Cold Spring Harbor Laboratory), Bill Studier (Brookhaven National Laboratory), and Nick Tonks (Cold Spring Harbor Laboratory).

This course is supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Allen, N., Ph.D., Stanford University School of Medicine, California
Blazek, D., Ph.D., University of California, San Francisco
Brito Lira, C., B.A., Universidade Estadual de Campinas, Brazil
Hess, H., B.S., Massachusetts Institute of Technology, Cambridge
Hoang, T., M.S., University of Bergen, Norway
Hundahl, C., Ph.D., Aarhus University, Denmark
Infante, R., B.S., University of Texas Southwestern Medical Center, Dallas
Kristensen, C., M.S., Poalis AS, Denmark
Kustka, A., Ph.D., Princeton University, New Jersey
Leibman, M., B.A., Harvard Medical School, Boston, Massachusetts
Luo, Y., B.S., State University of New York at Stony Brook
Martin, J., B.S., University of California, Irvine
Patil, R., Ph.D., Abbott Laboratory, Abbott Park, Illinois
Pearson, C., Ph.D., University of Colorado, Boulder
Peeters, T., M.S., Katholieke Universiteit Leuven, Belgium
Tian, L., Ph.D., The Samuel Roberts Noble Foundation, Ardmore, Oklahoma

SEMINARS

Burgess, R., University of Wisconsin, Madison: Introduction to protein purification, immunoaffinity chromatography; RNA polymerase–σ factor interactions and use of LRET-based assays for drug discovery and biochemistry.
Courey, A., University of California, Los Angeles: Groucho and Yan mediate the spreading of chromosomal silencing.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Protein crystallography introduction and demonstration; The crystal structure of Argonaute: The secret of life slicer.
Lazebnik, Y., Cold Spring Harbor Laboratory: Oncogenic transformation as a cause of apoptosis.
Lin, S.-H., M.D. Anderson Cancer Center/University of Texas, Houston: The bone marrow proteome as a discovery platform to understand prostate cancer progression.
Severinov, K., Waksman Institute, Rutgers University, Piscataway, New Jersey: Analyzing inhibitors of bacterial RNA polymerase.
Stillman, B., Cold Spring Harbor Laboratory: The role of ATP in replication origin recognition.
Studier, B., Brookhaven National Laboratory, Upton, New York: Protein production in the inducible *T7/E. coli* expression system.
Tonks, N., Cold Spring Harbor Laboratory: Signal transduction from the protein tyrosine phosphatase perspective.

Cell and Developmental Biology of *Xenopus*

April 9–19

INSTRUCTORS J. Heasman, **Children's Hospital Research Foundation, Cincinnati, Ohio**
C. Wylie, **Children's Hospital Research Foundation, Cincinnati, Ohio**

ASSISTANTS B. Birsoy, **Children's Hospital Research Foundation, Cincinnati, Ohio**
M. Kofron, **Children's Hospital Research Foundation, Cincinnati, Ohio**
T. Nakayama, **University of Virginia, Charlottesville**
J. Taylor, **Washington University, St. Louis, Missouri**

Xenopus is the leading vertebrate model for the study of gene function in development. The combination of lineage analysis, gene knockout strategies, experimental manipulation of the embryo, and genomic/bioinformatic techniques makes it ideal for studies on the molecular control of embryo patterning, morphogenesis, and organogenesis. The course combined intensive laboratory training with daily lectures from recognized experts in the field. Students learned both emerging technologies and classical techniques to study gene function in *Xenopus* development. An important element was the informal interaction between students and course faculty. Technologies covered included oocyte and embryo culture, lineage analysis and experimental manipulation of embryos, gain- and loss-of-function analyses using mRNAs and antisense oligos, whole-mount in situ hybridization, immunocytochemistry,

RT-PCR, and genomic/bioinformatic techniques, preparation of transgenic embryos, use of egg extracts to study the cell cycle and use of *Xenopus tropicalis* for genetic analyses. This course was designed for those new to the *Xenopus* field, as well as for those wanting a refresher course in the emerging technologies. The course was open to investigators from all countries. Additional lecturers in the 2005 course included Stefano Piccolo, Ray Keller, Aaron Zorn, Kristen Kroll, Betsy Pownall, Matt Kofron, Paul Krieg, and Hironon Funabiki.

This course is supported with funds provided by the National Institute of Child Health and Human Development, the National Science Foundation, and the Howard Hughes Medical Institute.

PARTICIPANTS

Babu, P., Ph.D., University of California, Livermore
Daniel, J., Ph.D., McMaster University, Hamilton, Canada
Darden, A., Ph.D., The Citadel, Charleston, South Carolina
Jean, S., B.S., Laval University, Quebec, Canada
Jelaso, A., Ph.D., Western Michigan University, Kalamazoo
Jorgensen, P., Ph.D., Harvard Medical School, Boston, Massachusetts
Kefalov, V., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Lee, W.M.K., Ph.D., Centre de Biologie du Development, Toulouse, France
Malone, J., M.S., The University of Texas, Arlington
Millaras, N., Ph.D., University of Virginia School of Medicine, Charlottesville
Nishanian, T., Ph.D., National Cancer Institute, Frederick, Maryland
Roose, M., M.S., Universitatsklinikum Essen, Germany
Susanto, J., B.S., University of Bath, United Kingdom
Tomlinson, M., B.S., The University of East Anglia, Norwich, United Kingdom
Watanabe, K., Ph.D., The University of Texas, Austin

SEMINARS

Funabiki, H., The Rockefeller University, New York: Cell cycle control and spindle formation in *Xenopus* egg extracts.
Heasman, J., Cincinnati Children's Hospital Research Foundation, Ohio: Maternal control of development.
Keller, R., University of Virginia, Charlottesville: Tissue movements in the early embryo.
Kofron, M./Zorn, A., Cincinnati Children's Hospital Research Foundation, Ohio: Bioinformatics and *Xenopus*.
Krieg, P., University of Arizona College of Medicine: Development of the cardiovascular.
Kroll, K., Washington University School of Medicine, St. Louis, Missouri: Making neurons in the early embryo.
Piccolo, S., University of Padua, Italy: Establishment of the primary germ layers.
Pownall, B., University of York, United Kingdom: Learning about cell signaling from *Xenopus tropicalis*.
Schriml, L., National Center for Biotechnology Information, Bethesda, Maryland: Bioinformatics and *Xenopus*.
Wylie, C., Cincinnati Children's Hospital Research Foundation, Ohio: Classical experiments in *Xenopus*.
Zorn, A., Cincinnati Children's Hospital Research Foundation, Ohio: Endoderm development.

Molecular Neurology and Neuropathology

June 8–14

INSTRUCTORS **S. Gandy,** Thomas Jefferson University, Philadelphia, Pennsylvania
J. Hardy, National Institutes of Health/NIA, Bethesda, Maryland
H. Orr, University of Minnesota, Minneapolis

This intensive 1-week discussion course offered successful applicants a unique opportunity to learn the latest concepts and methodologies associated with the study of human neurological disorders such as Alzheimer's disease, Parkinson's disease, and epilepsy. Participants discussed in detail the strengths and weaknesses of the accumulated experimental evidence underlying our current understanding of these diseases. Fundamental questions—such as how and why particular neurons die in certain disorders—were discussed in the context of identifying the best experimental approaches to finding answers, whether through the use of transgenic and/or lesion-induced mouse models, functional brain and/or cellular imaging, gain-/loss-of-function molecular and viral approaches, cellular transplantation, or a combination of these approaches. The course examined why many of these disorders share apparently common features—protein aggregation, specific vulnerability of certain classes of neurons, long incubation period—and discussed to what extent these features reflect common

pathological mechanisms. The course further explored how the underlying mechanisms in these disparate disorders were targeted for potential diagnostic and therapeutic gain. Extended seminars and discussion by a wide range of leading investigators further illuminated developments in this rapidly moving field. Participation in the course provided an essential conceptual and methodological framework for anyone intending to pursue rigorous research. Speakers this year included John Collinge (University College London), Mark Cookson (National Institute on Aging), Monica Driscoll (Rutgers, The State University of New Jersey), Katrina Gwinn-Hardy (NINDS), Michael Hutton (Mayo Clinic, Jacksonville), Mathias Jucker (Gerontology Research Center), Ron Kopito (Stanford University), Marcy MacDonald (Massachusetts General Hospital), James McNamara (Duke University Medical Center), Diane Murphy (NINDS), Henry Paulson (University of Iowa Hospital and Clinics), Peter Schofield (The Garvan Institute of Medical Research), Jerome Siegel (Veterans Affairs Greater L.A. Health Care System), and Maurice Swanson (University of Florida).

PARTICIPANTS

Adighibe, O., M.S., National Institutes on Aging, Bethesda, Maryland
Chang, T.Y., Ph.D., Dartmouth Medical School, Hanover, New Hampshire
Davis, R., Ph.D., State University of New York Upstate Medical University, Syracuse
Fung, H.-C., M.D., National Institutes of Health, Bethesda, Maryland
Gollamudi, S., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Horsford, D.J., Ph.D., National Institutes of Health, Bethesda, Maryland
Jain, S., B.S., National Institutes on Aging, Bethesda, Maryland
Kim, J., B.S., Mayo Clinic College of Medicine, Jacksonville, Florida
Klupsch, K., M.S., London Research Institute, United Kingdom
Lindemann, L., Ph.D., F. Hoffmann–La Roche Ltd., Basel, Switzerland
Marlowe, L., B.A., National Institutes of Health, Bethesda, Maryland
McFarlane, D., D.V.M., Atlantic Veterinary College, Charlottetown, Canada
Oyenuga, K.O., Ph.D., Philadelphia College of Pharmacy, Pennsylvania
Rasse, T., Ph.D., Max-Planck Society, Goettingen, Germany
Stewart, H., B.S., Umea University, Sweden
van de Leemput, J., M.S., National Institutes of Health, Bethesda, Maryland

SEMINARS

Collinge, J., University College London, United Kingdom: Molecular biology of prior propagation.
Cookson, M., NIA/NIH, Bethesda, Maryland: Parkinson's disease.
Driscoll, M., Rutgers, The State University of New Jersey, Piscataway: Genetic dissection of molecular mechanisms of necrosis in *C. elegans*.
Gandy, S., Thomas Jefferson University, Philadelphia, Pennsylvania: Amyloid and Alzheimer's disease.
Gwinn-Hardy, K., NINDS/NSC, Bethesda, Maryland: Clinical features of neurodegenerative disorders.
Hardy, J., NIA/NIH/Laboratory of Neurogenetics, Bethesda, Maryland: Genetic analysis in neurological diseases.
Hutton, M., Mayo Clinic Jacksonville, Florida: The Tauopathies: Genetics and pathobiology.
Jucker, M., Gerontology Research Center, Baltimore, Maryland: Cerebral amyloidoses.
Kopito, R., Stanford University, California: The cell biology protein aggregation diseases.
Macdonald, M., Massachusetts General Hospital, Charlestown: Huntington's disease.
McNamara, J., Duke University Medical Center, Durham, North Carolina: Epilepsy overview; Neurotrophines and epileltogenesis.
Murphy, D., NINDS/NIH, Rockville, Maryland: NIH grant process.
Orr, H., University of Minnesota, Minneapolis: SCA1, a neurodegenerative disease from gene to cellular pathway.
Paulson, H., University of Iowa: RNA interference and its application to neurobiological diseases.
Schofield, P., The Garvan Institute of Medical Research, Sydney, Australia: The inhibitory glycine receptor: Molecular studies of structure, function, and disease.
Siegel, J., Veterans Affairs Greater L.A. Health Care System, North Hills, California: Narcolepsy and the role of hypothalmic peptide hypocretin (orexin).
Swanson, M., University of Florida, Gainesville: The myotonic dystrophies; RNA-mediated disease.

Advanced Bacterial Genetics

June 8–28

INSTRUCTORS **K. Hughes,** University of Washington, Seattle
U. Jenal, University of Basel, Switzerland
K. Pogliano, University of California, San Diego

ASSISTANTS **S. Aung,** University of California, San Diego
B. Christen, University of Basel, Switzerland
S. Weissman, Children's Hospital and Regional Medical Center, Seattle, Washington

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 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. Speakers in the 2005 course included Dan Andersson (Uppsala University), Steve Busby (University of Birmingham), Victor DiRita (University of Michigan Medical School), John Kirby (Georgia Institute of Technology), Susan Lovett (Dana-Farber Cancer Institute), Anca Segall (San Diego State University), Gisela Storz (National Institute of Child Health and Human Development), Michael Surrette (University of Calgary), and Barry Wanner (Purdue University).

This course is supported with funds provided by the National Science Foundation.

PARTICIPANTS

Aertsen, A., Ph.D., Katholieke Universiteit Leuven, Belgium
Andrews, S., Ph.D., Lawrence Berkeley National Laboratory, California
Fernandes, A., Ph.D., Lisbon and University of Zurich, Oeiras, Portugal
Fineran, P., B.S., University of Cambridge, United Kingdom
Guberman, J., A.B., Princeton University, New Jersey
Gummesson, B., B.S., Goteborg University, Sweden
Haenni, M., M.C., University of Lausanne, Switzerland
Higashi, J., Ph.D., University of California, San Francisco
Jaeger, T., B.S., University of Konstanz, Germany
Jones, S., B.A., Baylor College of Medicine, Houston, Texas
Larsson, D., M.S., Uppsala University, Sweden
Laubacher, M., B.S., Pennsylvania State University, University Park
Rowland, S., B.S., University of Connecticut Health Center, Farmington
Shimizu, T., Ph.D., Harvard University, Cambridge, Massachusetts
Shinar, G., M.S., Weizmann Institute of Science, Rehovot, Israel
Zheng, J., M.S., University of Maryland, College Park

SEMINARS

Andersson, D., Uppsala University, Sweden: Where do new genes come from? Clues from studies of antibiotic resistance.
Busby, S., University of Birmingham, United Kingdom: Transcriptional regulation at complex bacterial promoters.
DiRita, V., University of Michigan Medical School, Ann Arbor: Genetic approaches to the study of bacterial pathogenesis.
Kirby, J., Georgia Institute of Technology, Atlanta: Chemosensory regulation of bacterial gene expression.
Lovett, S., Dana-Farber Cancer Institute, Boston, Massachusetts: *E. coli's* response to replication fork arrest.
Segall, A., San Diego State University, California: DNA repair, a new target for antibiotics: A journey from the esoteric to the practical.
Storz, G., National Institute of Child Health and Human Development, Bethesda, Maryland: The genes that were missed: An expanding universe of small RNAs and small CRFs.
Surrette, M., University of Calgary, Canada: Adaptations to polymicrobial life: Pathogen–normal flora interaction.
Wanner, B., Purdue University, W. Lafayette, Indiana: Red, CRIM, CAS, and FICkle (changeable) bacterial designer systems.

Ion Channel Physiology

June 8–28

INSTRUCTORS
M. Farrant, University College London, United Kingdom
M. Hausser, University College London, United Kingdom
N. Spruston, Northwestern University, Evanston, Illinois

COINSTRUCTORS
B. Clark, University College London, United Kingdom
J. Diamond, National Institutes of Health, Bethesda, Maryland
Z. Nusser, Institute of Experimental Medicine, Budapest, Hungary
I. Soltesz, University of California, Irvine

ASSISTANTS
T. Aman, Northwestern University, Chicago, Illinois
C. Foldy, University of California, Irvine
T. Jarsky, Northwestern University, Chicago, Illinois

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 the neurotransmitter at central and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, and (3) responded to neuromodulators with changes in functional properties. 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 centered on patch-clamp recording of ion channel activity in neurons in brain slice preparations. Different recording methods were used to examine in isolation the activity of specific voltage and ligund-gated ion channels and to investigate the contribution of specific ionic currents to the generation of neuronal activity. The advantages and disadvantages of each method, preparation, and recording technique were considered in relation to the specific scientific questions being asked. Admissions priority was given to students and postdocs with a demonstrated interest, specific plans, and a supportive environment to apply these techniques to a defined problem. Guest speakers in 2005 included Angel Alonso (McGill University), Bruce Bean (Harvard Medical School), Benoit Roux (Cornell University), Richard Horn (Jefferson Medical College), Jeffry Isaacson (University of California, San Diego), Dan Johnston (University of Texas), Rodolfo Llinas (New York University School of Medicine), Jeffrey Magee (LSUHSC), Sacha Nelson (Brandeis University), Karel Svoboda (HHMI/Cold Spring Harbor Laboratory), Tomoyuki Takahashi (University of Tokyo Graduate School of Medicine), and Rachel Wilson (Harvard Medical School).

This course is supported by the Howard Hughes Medical Institute.

PARTICIPANTS

Blankenship, A., B.A., University of California, San Diego
Branco, T.A., M.D., University College London, United Kingdom
Carrasquillo, Y., B.S., Baylor College of Medicine, Houston, Texas
Christian, C., B.A., University of Virginia, Charlottesville
Genoux, D., Ph.D., College de France, Paris, France
Jefferis, G., Ph.D., University of Cambridge, United Kingdom
Luikart, B., Ph.D., University of Texas Southwestern Medical School, Dallas
Macpherson, L., B.S., Scripps Research Institute, La Jolla, California
Matthews, E., B.S., Northwestern University, Chicago, Illinois
Ozkan, E., B.S., University of Texas Southwestern Medical School, Dallas
Pratt, K., Ph.D., Brown University, Providence, Rhode Island
Szabadics, J., Ph.D., University of Szeged, Hungary

SEMINARS

Alonso, A., McGill University, Montreal, Canada: Diversity of intrinsic excitability in the central nervous system.
Bean, B., Harvard Medical School, Boston, Massachusetts: Voltage-gated Na^+ channels; Na^+ channels and spontaneous firing.
Diamond, J., National Institutes of Health, Bethesda, Maryland: Glutamate receptors and synaptic excitation.
Horn, R., Jefferson Medical College, Philadelphia, Pennsylvania: Voltage-gated K^+ channels.
Isaacson, J., University of California, San Diego: Microcircuits.
Johnston, D., University of Texas, Houston: Ion channels and integration.
Llinas, R., New York University School of Medicine: Integration in neurons with intrinsic electrical attitude.
Magee, J., Louisiana State University Health Sciences Center, New Orleans: Hyperpolarization-activated cation channels.
Nelson, S., Brandeis University, Waltham, Massachusetts: Long-term synaptic plasticity.
Nusser, Z., Hungarian Academy of Sciences, Budapest, Hungary: Synaptic inhibition and interneuron diversity.
Roux, B., Cornell University, Ithaca, New York: The structure of K^+ channels.
Soltesz, I., University of California, Irvine: Endocannabincoid-mediated short-term plasticity.
Svoboda, K., Cold Spring Harbor Laboratory: Imaging methods for studying ion channels.
Takahashi, T., University of Tokyo Graduate School of Medicine, Japan: Voltage-gated Ca^{2+} channels.
Wilson, R., Harvard Medical School, Boston, Massachusetts: Intrinsic conductances and synaptic properties of *Drosophila* neurons.

Molecular Embryology of the Mouse

June 8–25

INSTRUCTORS	**B. Capel,** Duke University Medical Center, Durham, North Carolina **M. Shen,** UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey
COINSTRUCTORS	**D. Threadgill,** University of North Carolina, Chapel Hill **P. Trainor,** Stowers Institute for Medical Research, Kansas City, Missouri
ASSISTANTS	**D. Coveney,** University of North Carolina, Chapel Hill **D. Escalante-Alcalde,** Institute of Cellular Physiology-UNAM, Mexico **A. Lulianella,** Stowers Institute for Medical Research, Kansas City, Missouri **J. Rivera,** University of North Carolina, Chapel Hill

This intensive laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse development. Lectures provided the conceptual basis for contemporary research in mouse embryogenesis and organogenesis, and laboratory practicals provided extensive hands-on introduction to mouse embryo analysis. Experimental techniques covered included in vitro culture and manipulation of pre- and postimplantation embryos, embryo transfer, culture and

genetic manipulation of embryonic stem cells, production of chimeras by embryo aggregation and by ES cell injection, and transgenesis by pronuclear microinjection. In addition, this year's practicals featured increased emphasis on phenotypic analysis of mutants, including techniques of histology, in situ hybridization, immunohistochemistry, skeletal preparation, organ culture, and tissue recombination. Confirmed speakers for this year included Kathryn Anderson, Greg Barsh, Richard Behringer, Jeff Christiansen, Frank Constantini, Gordon Fishell, Kat Hadjantonakis, Tyler Jacks, Rudolf Jaenisch, Robin Lovell-Badge, Terry Magnuson, Anne McLaren, Andras Nagy, Tony Perry, Nadia Rosenthal, Janet Rossant, James Sharpe, Austin Smith, Davor Solter, Phillippe Soriano, Colin Stewart, Joseph Takahashi, Patrick Tam, Daniel Turnbull, and Rolf Zeller.

PARTICIPANTS

Brachner, A., M.S., Medical University of Vienna, Austria
Colon, M., Ph.D., University of Puerto Rico, San Juan
Cox, M., Ph.D., Mayo Clinic Arizona, Scottsdale, Arizona
Duncan, K., B.S., Harvard University, Cambridge, Massachusetts
Elso, C., Ph.D., Lawrence Livermore National Laboratory, California
Enciso, J., M.D., Baylor College of Medicine, Houston, Texas
Hamalainen, R., M.S., University of Helsinki, Finland
Hopyan, S., Ph.D., The Hospital for Sick Children, Toronto, Canada
Kanning, K., B.A., University of Washington, Seattle
Lecoin, L., Ph.D., Institut Curie, Orsay, France
Regard, J., Ph.D., University of California, San Francisco
Rodriquez, A., Ph.D., Wellcome Trust Sanger Institute, Hinxton, United Kingdom
Sears, K., Ph.D., University of Colorado Health Sciences Center, Aurora
Winter, J., Max-Planck Institute, Berlin, Germany

SEMINARS

Anderson, K., Memorial Sloan-Kettering Cancer Center, New York: Forward genetic screens.
Barsh, G., HHMI/Stanford University, California: Biology of pigmentation and its applications.
Behringer, R., The University of Texas/M.D. Anderson Cancer Center, Houston: Transgenics and insertional mutants.
Capel, B., Duke University, Durham, North Carolina: Intermediate mesoderm.
Capel, B., Duke University, Durham, North Carolina/Shen, M., UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey: Arrival, introductions, overview.
Costantini, F., Columbia University, New York: Kidney development and branching morphogenesis.
Fishell, G., The Skirball Institute, New York University Medical Center: Cellular and developmental genetic mechanisms underlying the establishment of cell fate in the brain.
Hadjantonakis, K., Columbia University, New York: Optical imaging.
Jacks, T., HHMI/Massachusetts Institute of Technology, Cambridge: Cancer models.
Jaenisch, R., Whitehead Institute/Massachusetts Institute of Technology, Cambridge: Epigenetics and stem cells.
Lovell-Badge, R., MRC National Institute for Medical Research: Sex determination.
Magnuson, T., University of North Carolina, Chapel Hill: Mouse genetics.
McLaren, A., University of Cambridge, United Kingdom: Germ cells.
Nagy, A., Samuel Lunenfeld Research Institute, Toronto, Canada: Gene targeting strategies.
Perry, T., RIKEN Center for Developmental Biology, Kobe, Japan: Nuclear reprogramming and oocyte activation.
Rosenthal, N., EMBL, Monterotondo-Scala, Italy: Cardiac organogenesis.
Rossant, J., Samuel Lunenfeld Research Institute, Toronto, Canada: Studying gene function without germ line transmission: Chimeras and siRNA.
Sharpe, J., MRC Human Genetics Unit, Edinburgh, United Kingdom: OPT microscopy and the Edinburgh mouse atlas.
Shen, M., UMNDJ–Robert Wood Johnson Medical School, Piscataway, New Jersey: Nodal signaling and embryo patterning.
Smith, A., University of Edinburgh, United Kingdom: Stem cells: Pluripotency and lineage restriction.
Solter, D., Max-Planck Institut für Immunobiologie, Freiburg, Germany: Fertilization and preimplantation development.
Soriano, P., Fred Hutchinson Cancer Research Center, Seattle, Washington: Functional analysis of signaling pathways/gene traps.
Stewart, C., National Cancer Institute, Frederick, Maryland: ES cells.
Takahashi, J., Northwestern University, Evanston, Illinois: Behavioral genetics.
Tam, P., Children's Medical Research Institute, Sydney, Australia: Introduction to mouse development; Gastrulation and formation of the body plan.
Threadgill, D., University of North Carolina, Chapel Hill: Quantitative traits.
Trainor, P., Stowers Institute for Medical Research, Kansas City, Missouri: *Hox* genes and the body axis.
Turnbull, D., New York University Medical Center: Ultrasound/MRI imaging.
Zeller, R., EMBL, Heidelberg, Germany: Limb development.

DNA Microarray Applications

June 14–21

INSTRUCTORS	**A. Khodursky,** University of Minnesota, St. Paul **J. Lieb,** University of North Carolina, Chapel Hill **C. Murphy,** University of California, San Francisco
ASSISTANTS	**S. Hanlon,** University of North Carolina, Chapel Hill **A. Hastings,** Molecular Devices Corporation, Union City, California **P. Killion,** University of Texas, Austin **M. Llinas,** Princeton University, New Jersey **P. Mieczkowski,** Duke University, Chapel Hill, North Carolina **D. Sangurdekar,** University of Minnesota, Minneapolis

DNA microarrays are simple, inexpensive, and versatile tools for experimental exploration of genome structure, gene expression programs, gene function, and cell and organismal biology. In this hands-on 8-day course, students were introduced to various applications of DNA microarray technology. Using whole-genome spotted DNA microarrays as a platform, students learned to design and carry out the procedures required for DNA microarray hybridization experiments, including fluorescent probe preparation, nucleic acid amplification, microarray manipulation, image acquisition and processing, visualiza-

tion and interpretation of high-dimensional transcriptional data, and data storage and retrieval. In addition to learning the techniques involved in microarray experiments, they also discussed the basics of multivariate data analysis, the basics of statistical inference and classification, and, most importantly, biological interpretation of microarray experiments.

Experimental applications covered in the course included systematic studies of global gene expression programs, inferring gene function using microarrays, genome-wide DNA-protein interactions, and measuring changes in gene-copy number. Guest lecturers presented the state of the art in new technology, experimental applications, and interpretation of genome-wide data sets from a systems biology perspective. Students who complete this course should be equipped with the practical and theoretical skills to introduce or apply DNA microarray technology at their own laboratories or institutions. Speakers this year included Patrick Brown (Stanford University Medical Center), Elva Diaz (University of California, Davis), Gavin Sherlock (Stanford University), Damian Verdnik (Axon Instruments), and David Wang (Washington University School of Medicine).

PARTICIPANTS

Ahlgren, S., Ph.D., Children's Memorial Research Center, Chicago, Illinois
Braby, C., Ph.D., Monterey Bay Aquarium Research Institute, Moss Landing, California
Bryant, P., B.M., B.C., Murdoch Children's Research Institute, Parkville, Australia
Connelly, J., Ph.D., Duke University, Durham, North Carolina
Hamilton, A., Ph.D., Lawrence Livermore National Laboratory, California
Huang, Z.J., Ph.D., Cold Spring Harbor Laboratory
Jung, W.H., Ph.D., University of British Columbia, Vancouver, Canada
Kanae, I.-A., Ph.D., Cold Spring Harbor Laboratory
Kinney, J., B.A., Princeton University, New Jersey
Kundzewicz, A., M.S., University of Lausanne, Switzerland
Nikic, S., M.S., Imperial College, London, United Kingdom
Rehfuss, M., Ph.D., University of California, Davis
Segal, E., Ph.D., The Rockefeller University, New York
Swigonova, Z., Ph.D., University of Pittsburgh, Pennsylvania
Tilley, P., M.D., Provincial Laboratory for Public Health, Calgary, Canada
Vaidyanathan, R., Ph.D., Epicentre Technologies, Madison, Wisconsin
Waisberg, M., M.S., Universidade Federal de Minas Geralis, Belo Horizonte, Brazil
Wang, Q., Ph.D., Dana-Farber Cancer Institute/Harvard Medical School, Boston, Massachusetts
Wyatt, S., Ph.D., Ohio University, Athena
Zhu, Y., Ph.D., East Carolina University, Greenville, North Carolina

SEMINARS

Brown, P., Stanford University Medical School, California: Regulation of Alzheimer amyloid precursor trafficking and processing.
Diaz, E., University of California, Davis: R and bioconductor for microarray data analysis.
Hanlon, S., University of North Carolina, Chapel Hill: ChIP-chip experimental setup; Analysis of ChIP-chip data.
Hastings, A., Axon Instruments, Union City, California: Data analysis with acuity; Microarray scanning and first-pass analysis with Genepix Pro.
Khodursky, A., University of Minnesota, St. Paul: Statistical primer for microarray data analysis; Statistical design and analysis of microarray data #2.
Killion, P., University of Texas, Austin: The why and how of experiment submission using the longhorn array database; Data analysis of gene experiments using LAD.
Llinas, M., Princeton University, New Jersey: Dissecting the developmental transcriptome of *Plasmodium*.
Mieczkowski, P., University of North Carolina, Chapel Hill: Data analysis for aCGH experiments.
Murphy, C., Princeton University, New Jersey: Experimental design from a biologist's point of view.
Segal, E., The Rockefeller University, New York: Probabilistic: Models for inferring gene regulatory networks.
Sherlock, G., Stanford University Medical School, California: Introduction to DNA microarray technology; Principles of microarray data analysis.
Wang, D., Washington University, St. Louis, Missouri: Viral detection and discovery using DNA microarrays.
Wolfinger, R., SAS Institute, Inc., Cary, North Carolina: A workflow for array data analysis.

Structure, Function, and Development of the Visual System

June 16–29

INSTRUCTORS **B. Chapman,** University of California, Davis
W.M. Usrey, University of California, Davis

ASSISTANT **H. Allito**, 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 wish 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; role of patterned neuronal activity in the development of central visual pathways; and molecular mechanisms of development and plasticity in the visual system. Speakers in the 2005 course included Dora Angelaki (Washington University School of Medicine), Mark Bear (Massachusetts Institute of Technology/HHMI), Tobias Bonhoeffer (Max-Planck Institute of Neurobiology), Ken Britten (University of California, Davis), Matteo Carandini (The Smith-Kettlewell Eye Research Institute), E.J. Chichilnisky (The Salk Institute), Dennis

Dacey (University of Washington, Seattle), Marla Feller (University of California, San Diego), David Fitzpatrick (Duke University), Karl Geggenfunter (GieBen University), William Guido (Louisiana State University Medical Center), Takao Hensch (RIKEN Brain Science Institute), Judith Hirsch (University of Southern California, Los Angeles), Richard Krauzlis (Salk Institute for Biological Studies), John Maunsell (Baylor College of Medicine), J. Anthony Movshon (New York University), Jay Neitz (Medical College of Wisconsin), John Reynolds (The Salk Institute), Michael Shadlen (University of Washington), S. Murray Sherman (University of Chicago), Lawrence Snyder (Washington University School of Medicine), Peter Sterling (University of Pennsylvania), and Leland Stone (NASA Ames Research Center).

This course is supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Calamusa, M., B.S., Institute of Neuroscience, CNR, Pisa, Italy
de Labra, C., Ph.D., University of La Goruna, Spain
El-Shamayleh, Y., B.A., New York University
Graf, A., Ph.D., New York University
Hall, A., B.S., University of Texas, Dallas, Richardson
Kuchenbecker, J., B.S., Medical College of Wisconsin, Milwaukee
Lovejoy, L., B.S., The Salk Institute for Biological Studies, La Jolla, California
Lui, L., B.S., Monash University, Victoria, Australia
Mancuso, K., B.S, Medical College of Wisconsin, Milwaukee
Nauhaus, I., B.S, University of California, Los Angeles
Puri, M., B.S., University of California, Davis
Restani, L., Ph.D., Scuola Normale Superiore, Pisa, Italy
Sher, S., Ph.D., University of California, Santa Cruz
Speer, C., B.S., University of California, Davis
Thaler, L., B.A., The Ohio State University, Columbus
Troncoso, X., B.S., Barrow Neurological Institute, Phoenix, Arizona
Varela-Castro, C., Ph.D., University of Chicago, Illinois
Watson, T., B.P., University of Sydney, Australia

SEMINARS

Angelaki, D., Washington University School of Medicine, St. Louis, Missouri: Visual and vestibular interactions in a complex environment.
Bear, M., HHMI/Massachusetts Institute of Technology, Cambridge: How monocular deprivation shifts ocular dominance in visual cortex.
Bonhoeffer, T., Max-Planck Institut für Neurobiologie, Muenchen-Martinsried, Germany: Functional architecture of the primary visual cortex.
Britten, K., University of California, Davis: Neural correlates of heading and self-locomotion.
Carandini, M., Smith-Kettlewell Eye Research Institute, San Francisco, California: Receptive fields and suppressive fields in the early visual system.
Chapman, B., University of California, Davis: How the visual system got its stripes.
Chichilnisky, E.J., The Salk Institute, La Jolla, California: Ensemble coding of visual information in primate retina.
Dacey, D., University of Washington, Seattle: Ganglion cell diversity and function.
Feller, M., University of California, San Diego: The how and why of retinal waves.
Fitzpatrick, D., Duke University, Durham, North Carolina: Columns, connections, and representations: Probing the functional architecture of the primary visual cortex.
Gegenfurtner, K., GieBen University, Germany: Signals for speed perception and smooth pursuit eye movements.
Guido, B., Louisiana State University Medical Center: Cellular mechanisms underlying the remodeling of developing retinogeniculate connections.
Hensch, T., Brain Science Institute, Wako-shi, Japan: Molecular mechanisms of ocular domainance plasticity.
Hirsch, J., University of Southern California, Los Angeles: Cellular mechanisms for visual responses in the LGN and primary visual cortex.
Krauzlis, R., The Salk Institute, San Diego, California: Coordination of voluntary eye movements.
Maunsell, J., HHMI/Baylor College Medicine, Houston, Texas: The effects of attention in extrastriate visual cortex.
Movshon, T., New York University: Motion processing in extrastriate cortex.
Neitz, J., Medical College of Wisconsin, Milwaukee: Evolution of color vision.
Reynolds, J., The Salk Institute, La Jolla, California: Attentional control of visual processing in the macaque.
Shadlen, M., University of Washington, Seattle: Decision making in extrastriate cortex.
Sherman, M., University of Chicago, Illinois: Cell and circuit properties of the thalamus.
Snyder, L., Washington University School of Medicine, St. Louis: Motor-specific regulation of visual signals in the extrastriate visual cortex?
Sterling, P., University of Pennsylvania, Philadelphia: Efficient circuits from stochastic synapses; Why the retina uses parallel pathways.
Stone, L., NASA Ames Research Center, Moffiett Field, California: Visual signals linking eye movements and perception.
Usrey, M., University of California, Davis: Dynamic properties of neural circuits for vision.

Statistics of Microarrays and Related Biological Data

June 21–26

INSTRUCTORS
J. Cabrera, Rutgers University, Piscataway, New Jersey
V. Carey, Harvard University, Boston, Massachusetts
M. Reimers, National Institutes of Health, Bethesda, Maryland

ASSISTANT
J. Gentry, Massachusetts General Hospital, Charlestown, Maryland

High-throughput biology, epitomized by the rapid growth of DNA microarray-based experiments, is rapidly generating enormous observation sets. Biologists seeking to make sense of this growing body of data need to have a firm grasp of statistical methodology. This short residential course was designed to build competence in statistical and related quantitative methods for the analysis of high-throughput biological and biochemical data, from which meaningful inferences about biological processes can be drawn.Topics included R minitutorial; bioconductor; review of multivariate statistics; discrimination and classification; expression arrays including experimental design, array design, quality control, normalization, and probe-level analysis for spotted arrays and for Affymetrix(TM) chips; exploratory analysis and tests of significance; and leveraging annotations (e.g., gene ontology).

The course was offered as a supplement to the 2005 course on DNA microarray applications. Preference was given to students accepted into that course, but the supplemental course also attracted biologists with a strong interest or background in quantitative thinking, as well as students with a background in mathematics, physics, and related disciplines who already demonstrated a firm grasp of major biological concepts and were interested in focusing their efforts on large biological data sets such as whole-genome or expression microarray data.

PARTICIPANTS

Connelly, J., Ph.D., Duke University, Durham, North Carolina
Contreras-Morei, B., Ph.D., Universidad Autonoma Nacional de Mexico (UNAM), Cuernavaca
Cortes, D., B.S., Virginia Tech University, Blacksburg
Einecke, G., M.D., University of Alberta, Edmonton, Canada
Gao, H., Ph.D., Karolinska Institutet, Huddinge, Sweden
Hamilton, A., B.A., Lawrence Livermore National Laboratory, California
Konieczka, J., B.S., University of Arizona, Tucson
Kundzewicz, A., M.S., University of Lausanne, Switzerland
Krasnitz, A., Ph.D., Cold Spring Harbor Laboratory
Pai, D., M.S., Cold Spring Harbor Laboratory
Raby, B., M.D., Harvard Medical School, Boston, Massachusetts
Swigonova, Z., Ph.D., University of Pittsburgh, Pennsylvania
Tan, R., M.S., University of Alabama, Birmingham
Torres, K., B.S., IIT Research Institute, Chicago, Illinois
Vaidyanathan, R., Ph.D., Epicentre Technologies, Madison, Wisconsin
Waisberg, M., M.D., Universidade Federal de Minas Gerals, Belo Horizonte, Brazil
Wang, L., Ph.D., Vanderbilt University, Nashville, Tennessee
Wyatt, S., Ph.D., Ohio University, Athens
Zhu, Y., Ph.D., East Carolina University, Greenville, North Carolina

SEMINARS

Cabrera, J., Rutgers University, Piscataway, New Jersey: Exploratory methods.
Cabrera, J., Rutgers University, Piscataway, New Jersey/Carey, V., Harvard University, Boston, Massachusetts: cDNA workflow.
Carey, V., Harvard University, Boston, Massachusetts: Bioconductor introduction; Machine learning.
Reimers, M., National Institutes of Health, Bethesda, Maryland: Affy workflow.
Reimers, M., National Institutes of Health, Bethesda, Maryland/Cabrera, J., Rutgers University, Piscataway, New Jersey: Low-level analysis and QC; Formal inference/siggenes; GO and functional inference.
Reimers, M., National Institutes of Health, Bethesda, Maryland/Cabrera, J., Rutgers University, Piscataway, New Jersey/Carey, V., Harvard University, Boston, Massachusetts: Introduction to the course.

Molecular Approaches in Plant Science

July 1–21

INSTRUCTORS
J. Bender, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland
L. Hobbie, Adelphi University, Garden City, New York
H. Ma, Pennsylvania State University, University Park
S. McCormick, Plant Gene Expression Center, Albany, California

ASSISTANTS
B. Feng, Pennsylvania State University, University Park
S. Johnson-Brousseau, University of California, Berkeley
A. Wijeratne, Pennsylvania State University, University Park

This course provided an intensive overview of topics in plant physiology and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from *Arabidopsis thaliana* and other plants and provided an introduction to current methods used in plant molecular biology. It was designed for scientists with some experience in molecular techniques or in plant biology who wish to work with *Arabidopsis* and other plants using the latest technologies in genetics and molecular biology. 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 (such as development of flowers, leaves, roots, male and female gametophytes, meristems, the vascular tissue, and embryos), perception of light and photomorphogenesis, and synthesis, function, and perception of hormones. Lectures describing bioinformatics tools available to the plant community and the resources provided by plant genome projects were also included. Speakers provided overviews of their fields, followed by in-depth discussions of their own work. The laboratory ses-

sions provided an introduction to important techniques currently used in plant research. The laboratory sessions included studies of plant development, microsopic techniques including Normarski/DIC, fluorescence, and SEM, enhancer traps, analysis of pollen development and meiosis, analysis of quantitative traits using natural variation, mutant analysis, histochemical staining, transient gene expression, applications of green fluorescent protein fusions, protein interaction and detection, proteomics approaches, transcription profiling, and techniques commonly used in genetic and physical mapping. The course also included several short workshops on important themes in genetics. Invited speakers for the 2005 course included Jody Banks (Purdue University), Gloria Coruzzi (New York University), Savithramma Dinesh-Kumar (Yale University), Jennifer Fletcher (USDA-ARS/University of California, Berkeley), Ueli Grossniklaus (University of Zurich Switzerland), Roger Hangarter (Indiana University), John Harada (University of California, Davis), Paul Herzmark (University of California, San Francisco), Thomas Jack (Dartmouth College), David Jackson (Cold Spring Harbor Laboratory), Teh-Hui Kao (Pennsylvania State University), Jiangming Li (University of Michigan), Wolfgang Lukowitz (Cold Spring Harbor Laboratory), Jocelyn Malamy (University of Chicago), Timothy Nelson (Yale University), Thomas Nuhse (The Sainsbury Laboratory, UK), Neil Olszewski (University of Minnesota), Robert Pruitt (Purdue University), Eric Richards (Washington University), G. Eric Schaller (Dartmouth College), and Marja Timmermans (Cold Spring Harbor Laboratory).

PARTICIPANTS

Baker, R., B.A., University of Colorado, Boulder
Brudenell, L., B.S., University of Bath, United Kingdom
Chen, Y., Ph.D., Agricore United, Manitoba, Canada
Chow, K.-S., Ph.D., Rubber Research Institute of Malaysia, Selangor
Conrad, P., Ph.D., State University of New York, Plattsburgh
Fick, J., B.S., University of Virginia, Charlottesville
Fuell, C., Ph.D., Institute of Food Research, Norwich, United Kingdom
Greenberg, N., B.A., University of Colorado, Boulder
Gutzat, R., M.S., Swiss Federal Institute of Technology, Zurich, Switzerland
Hou, Y., M.S., Iowa State University, Ames
Jensen, J., M.S., The Royal Veterinary and Agricultural University, Frederiksberg, Denmark
Jugulam, M., Ph.D., University of Western Ontario, Guelph, Canada
Kondo, M., B.S., The University of Tokyo, Japan
White, M., M.S., University of Wisconsin, Madison

SEMINARS

Banks, J., Purdue University, W. Lafayette, Indiana: Gametophytes and evolution.
Bender, J., Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland: Epigenetics II.
Coruzzi, G., New York University: Systems biology.
Dinesh-Kumar, S., Yale University, New Haven, Connecticut: Plant-pathogen interactions.
Fletcher, J., USDA-ARS/University of California, Berkeley: Inflorescence.
Grossniklaus, U., University of Zurich, Switzerland: Female gametophytes.
Hangarter, R., Indiana University, Bloomington: Light responses.
Harada, J., University of California, Davis: Seed development.
Herzmark, P., University of California, San Francisco: Microscopy lecture and lab.
Hobbie, L., Adelphi University, Garden City, New York: Auxin signaling.
Jack, T., Dartmouth College, Hanover, New Hampshire: Flower development.
Jackson, D., Cold Spring Harbor Laboratory: Meristems.
Kao, T.-H., Pennsylvania State University, University Park: Self-incompatibility.
Li, J., University of Michigan, Ann Arbor: Brassinosteroids.
Lukowitz, W., Cold Spring Harbor Laboratory: Embryo development.
Ma, H., Pennsylvania State University, University Park: Plant anatomy; Genetics I: Mutagenesis; Microsporogenesis; Evolution of flower development.
Malamy, J., University of Chicago, Illinois: Roots.
McCormick, S., Plant Gene Expression Center, Albany, California: Pollen development.
Nelson, T., Yale University, New Haven, Connecticut. Vascular development.
Nuhse, T., John Innes Centre, Norwick, United Kingdom: Proteomics.
Olszewski, N., University of Minnesota, St. Paul: GA signaling.
Pruitt, R., Purdue University, W. Lafayette, Indiana: Non-Mendelian Inheritance.
Richards, E., Washington University, St. Louis, Missouri: Epigenetics I.
Schaller, E., Dartmouth College, Hanover, New Hampshire: Ethylene.
Timmermans, M., Cold Spring Harbor Laboratory: Maize shoot patterning.

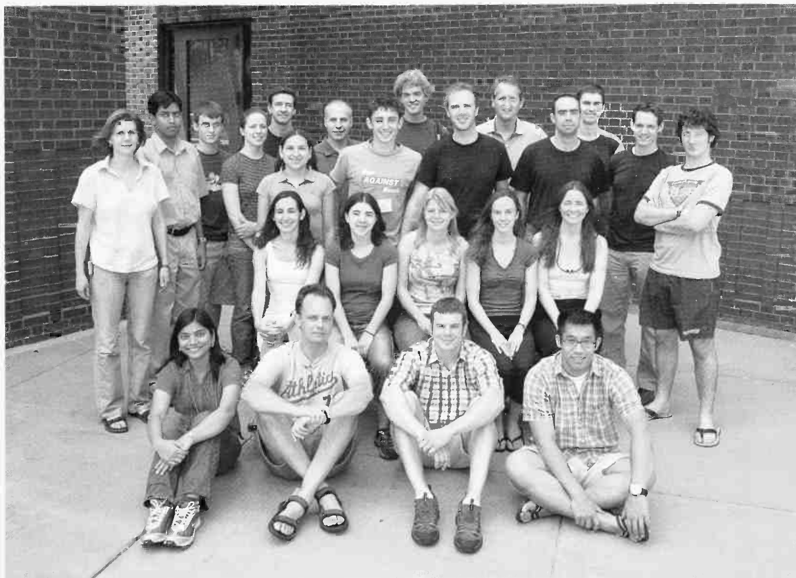
Neurobiology of *Drosophila*

July 1–21

INSTRUCTORS
R. Baines, University of Warwick, Coventry, United Kingdom
G. Bashaw, University of Pennsylvania, Philadelphia
S. Waddell, University of Massachusetts Medical School, Worcester

ASSISTANTS
D. Garbe, University of Pennsylvania, Philadelphia
A. Keene, University of Massachusetts Medical School, Worcester
B. Leung, University of Massachusetts Medical School, Worcester
D. Wright, 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 3-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 are 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 whole grain, 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 Marcus Allen (University of Kent), Vanessa Auld (University of British Columbia), Justin Blau (New York University), Akira Chiba (University of Illinois), Josh Dubnau (Cold Spring Harbor Laboratory), Daniel Eberl (University of Iowa), Donald Gailey (California State University, Hayward), Tanja Godenschwege (University of Massachusetts), Stephen Goodwin (University of Glasgow), Gregory Jefferis (University of Cambridge), Edward Kravitz (Harvard Medical School), Susana Lima (Cold Spring Harbor Laboratory), Greg Macleod (University of Arizona), Paul Shaw (Washington University School of Medicine), James Skeath (Washington University School of Medicine), Bryan Stewart (University of Toronto), Bruno van Swinderen (Neurosciences Institute), Tanya Wolff (Washington University School of Medicine), Bing Zhang (University of Texas, Austin), and Karen Zito (Cold Spring Harbor Laboratory).

PARTICIPANTS

Adolph, S., M.S., University of Copenhagen, Denmark
Bustamante, E., B.S., Stanford University, California
Dason, J., M.S., University of Toronto, Canada
Dixit, R., M.S., National Centre for Biological Sciences, Bangalore, India
Douglas, S., Ph.D., University of Toronto at Mississauga, Canada
Edwards, A., B.S., North Carolina State University, Raleigh
Fernandez, M.d.I.P., Licentiate, Biotechnology, Leloir Institute Foundation, Buenos Aires, Argentina
Haines, N., Ph.D., University of Toronto at Scarborough, Toronto, Canada
Kaiser, M., B.S., The University of Manchester, United Kingdom
Leiss, F., M.S, Max-Planck Institute of Neurobiology, Martinsried, Germany
Logan, M., Ph.D., University of Utah, Salt Lake City
Neely, G., Ph.D., Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austria

SEMINARS

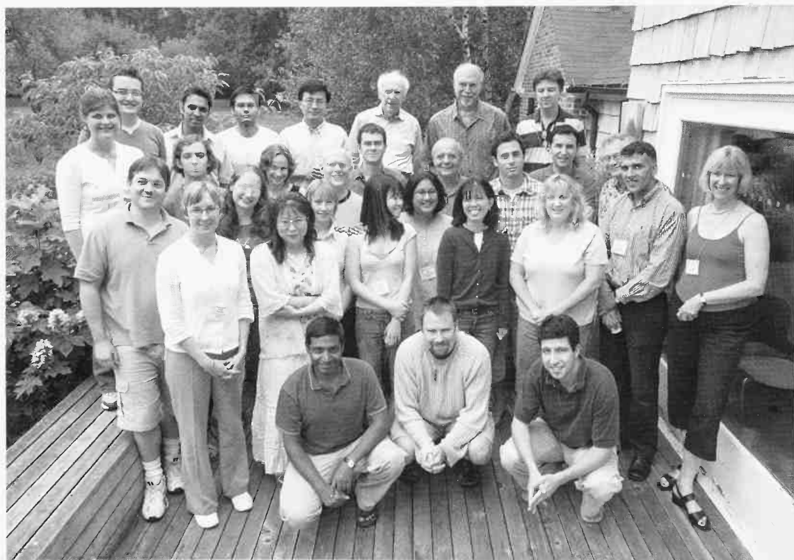
Allen, M., University of Kent, United Kingdom: Physiology of the giant fiber system.
Auld, V., University of British Columbia, Vancouver, Canada: Bioinformatics 101; Gilal development.
Baines, R., University of Warwick, United Kingdom: Synaptic plasticity; CNS electrophysiology.
Bashaw, G., University of Pennsylvania, Philadelphia: Axon guidance; Fly genetics 101.
Blau, J., New York University: Circadian rhythm.
Chiba, A., University of Illinois, Urbana: Dendritic development.
Dubnau, J., Cold Spring Harbor Laboratory: Long-term memory.
Eberl, D., University of Iowa, Iowa City: Hearing and smell.
Gailey, D., California State, Hayward: *Drosophila* courtship.
Godenschwege, T., University of Massachusetts, Amherst: Giant fiber system.
Goodwin, S., University of Glasgow, United Kingdom: Fruitless and control of sexual behavior.
Jefferis, G., Cambridge University, United Kingdom: Olfactory system development; Fly genetics 102.
Kravitz, E., Harvard Medical School, Boston, Massachusetts: Aggression.
Lima, S., Cold Spring Harbor Laboratory: Remote control of fly behavior.
Macleod, G., University of Arizona, Tucson: Imaging neural activity.
Shaw, P., Washington Medical School, St. Louis, Missouri: Sleep.
Skeath, J., Washington University, St. Louis, Missouri: Neuronal identity.
Stewart, B., University of Toronto, Canada: Electrophysiology 101; Synaptic release.
Van Swinderen, B., Neuroscience Institute, San Diego, California: Visual learning.
Waddell, S., University of Massachusetts Medical School, Worcester: Introduction; Olfactory learning and memory.
Wolff, T., Washington University, St. Louis, Missouri: Visual system.
Zhang, B., University of Texas, Austin: The Fly NMJ.
Zito, K., Cold Spring Harbor Laboratory: Light and confocal microscopy.

Workshop on Schizophrenia and Related Disorders

July 6–19

INSTRUCTORS **P. Harrison**, University of Oxford, United Kingdom
Z.J. Huang, Cold Spring Harbor Laboratory
J. Lieberman, Columbia University, New York
J. Lisman, Brandeis University, Waltham, Massachusetts
D. Stewart, Cold Spring Harbor Laboratory
D. Weinberger, NIMH/Neuroscience Center, Bethesda, Maryland

Recent progress in genetics, developmental neurobiology, and systems neuroscience presents an unprecedented opportunity to study the biological basis of this class of elusive diseases. This 2-week workshop addressed four main broad themes: clinical descriptions of schizophrenia and related disorders and current treatments; emerging clues from genetics and genomics of psychiatric disorders; current perspectives in brain development; and neuroanatomy and (mal)function of circuits and networks. Discussion of the four themes was led by moderators, who had individual responsibility for the individual workshop sections. The workshop included two seminars per day (morning and late afternoon/evening), although some days featured one or three talks depending on topic. Modules, moderators, and speakers included Clinical Background: History, Symptoms/Phenotypes, Treatments (Lieberman); Genetics, Genomics, and Epigenetics (Harrison/Weinberger); Development and Function of Neural Circuits (Huang); and Neural Systems, Cognition, and Psychosis (Lisman).



This workshop was supported with funds provided by the Oliver Grace Fund, and additional support for a limited number of U.K. student scholarships (including travel) was provided by the Medical Research Council.

PARTICIPANTS

Arana, F., M.S., University of Cambridge, United Kingdom
Baker, J., M.S., Washington University School of Medicine, St. Louis, Missouri
Barkus, E., Ph.D., University of Manchester, United Kingdom
Basu, A., Ph.D., Harvard Medical School, Belmont, Massachusetts
Buonanno, A., Ph.D., National Institutes of Health, Bethesda, Maryland
Chen, Y.-J., M.S., Columbia University, New York
Chubb, J., M.S., University of Edinburgh, United Kingdom
Corlett, P., M.S., Cambridge University, United Kingdom
Cressman, V., Ph.D., New York State Psychiatric Institute
Fejgin, K., M.S., Goteborg University, Sweden
Flinn, L., M.S., University of Washington, Seattle
Gilleen, J., M.S., Institute of Psychiatry, London, United Kingdom
Haggarty, S., Ph.D., Broad Institute of Harvard/Massachusetts Institute of Technology, Cambridge, Massachusetts
Hall, J., Ph.D., University of Edinburgh, United Kingdom
Kumra, S., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Menzies, L., M.S., University of Cambridge, United Kingdom
Morris, J., Ph.D., Northwestern University, Chicago, Illinois
Ozaki, M., Ph.D., Waseda University, Tokyo, Japan
Petryshen, T., Ph.D., Broad Institute of Harvard/Massachusetts Institute of Technology, Cambridge, Massachusetts
Raghavachari, S., M.S., Duke University Medical Center, Durham, North Carolina
Sahay, A., M.S., Columbia University, New York
Schobel, S., Ph.D., New York State Psychiatric Institute, New York
Shriki, O., Ph.D., Weizmann Institute of Science, Rehovot, Israel
Tinsley, M., Ph.D., University of California, Los Angeles

SEMINARS

Behrendt, R.-P., The Retreat Hospital, York, United Kingdom: Thalamus and hallucinations in schizophrenia.
Bell, C., Oregon Health Science University, Beaverton: Review of cortical circuits underlying corollary discharge: Role of the thalamus; Possible connection to the Llinas hypothesis; Mechanisms by which expected sensory signals are cancelled: Known mechanisms in the electric fish.
Cox, D., Perlegen Science, Mountain View, California: Human genetic variation and common human disease.
Coyle, J., Harvard University/McLean Hospital, Belmont, Massachusetts: Glutamate hypothesis.
Davis, K., Mount Sinai School of Medicine, New York: Oligodendroglia and myelination in schizophrenia.
Falls, D., Emory University, Atlanta, Georgia: Neuregulin.
Ford, J., Yale University, New Haven, Connecticut: Study of dysfunctional corollary discharge in schizophrenia as studied by fMRI and EEG coherence.
Frank, M., University of Colorado, Boulder: Normal function of the basal ganglia and reward systems; Model of set-shifting deficits in schizophrenia.
Goldberg, T., National Institutes of Mental Health, Bethesda, Maryland: Cognitive deficits.
Greenspan, R., The Neuroscience Institute, San Diego, California/A. Klar, National Institutes of Health, Frederick, Maryland: Handedness, laterality, and schizophrenia discussion.
Heckers, S., Harvard Medical School, McLean Hospital, Belmont, Massachusetts: Hippocampus in s chizophrenia.
Huang, Z.J., Cold Spring Harbor Laboratory: Construction of GABAergic circuits and synapses.
Javitt, D., Nathan Kline Institute, Orangeburg, New York: The biophysical basis of evoked potentials abnormal in schizophrenia: The involvement of the NMDA channel.
Jones, P., University of Cambridge, United Kingdom: Development-epidemiology.
Kapur, S., University of Toronto, Canada: Pathophysiology.
Karayiorgou, M., The Rockefeller University, New York: Genetic animal models of schizophrenia.
Kinsbourne, M., The New School, New York: Origins of voices: Exploring our conscious and unconscious selves.
Laruelle, M., Columbia University, New York: Dopamine.
Law, A., The University of Oxford, United Kingdom: Neuregulin transcription and posttranscriptional regulation.
Levitt, P., Vanderbilt University, Nashville, Tennessee: Development of cortical circuitry and disorders of information processing: Autism and schizophrenia.
Lewis, D., University of Pittsburgh, Pennsylvania: GABA.
Lisman, J., Brandies University, Waltham, Massachusetts:

The hippocampus, prefrontal cortex, basal ganglia, and VTA: An integrated system that underlies some of the cognitive deficits of schizophrenia; The loop between the hippocampus and the dopaminergic neurons of the VTA: Hippocampal hyperactivity in schizophrenia.

Llinas, R., New York University: Thalamocortical dysrhythmia.

Mathalon, D., Yale University, New Haven, Conneticut: Corollary discharge mechanism: How it works and why its dysfunction may explain auditory hallucinations; Abnormalities in corollary discharge as measured by auditory sensory event-related brain potentials; Role of the N1 component.

McGuire, P., Institute of Psychiatry, London, United Kingdom: Understanding the mechanisms of hallucinations.

McKay, R., National Institutes of Health, Bethesda, Maryland: Constructing the dopaminergic brain.

McKnight, S., University of Texas Southwestern Medical Center, Dallas: Transcription factors, mouse models.

Miller, E., Massachusetts Institute of Technology, Cambridge: Normal function of the PFC: Goal-directed behavior.

Mirnics, K., University of Pittsburgh, Pennsylvania: Genomic studies of schizophrenia: The good, the bad and the ugly.

O'Donnell, P., Albany Medical College, New York: Hypofrontality: Abnormalities in the accumbens/PFC/hippocampal/VTA system in schizophrenia; Normal function of accumbens.

O'Donovan, M., University of Wales, Cardiff, United Kingdom: Overview of schizophrenia genetics: Concepts, methods, current status.

Petronis, A., Center for Addiction and Mental Health, Toronto, Canada: Epigenetics and psychiatric diseases.

Porteous, D., University of Edinburgh, United Kingdom: DISC-1.

Rakic, P., Yale University, New Haven, Connecticut: Neurogenesis, lineage, migration, evolution?

Rapoport, J., National Institutes of Mental Health, Bethesda, Maryland: Watch normal and abnormal brains grow: Childhood onset schizophrenia.

Role, L., Columbia University, New York: Neuregulin: Synpatic physiology; Nicotinic acetycholin receptor signaling in schizophrenia.

Sebat, J., Cold Spring Harbor Laboratory: Gene copy-number variation in human disease.

Shergill, S., Institute of Psychiatry, London, United Kingdom: Review of psychological evidence for abnormalities in sense of self; Theory of mind.

Skuse, D., University College London, United Kingdom: Neurodevelopmental perspectives on genetics and cognition.

Straub, R., National Institutes of Mental Health, Bethesda, Maryland: Dysbindin and muted-susceptibility genes in the BLOC-1 complex.

Walsh, C., Harvard University, Cambridge, Massachusetts: Development and evolution of neocortex discussion.

Advanced Techniques in Molecular Neuroscience

July 6–21

INSTRUCTORS
J. Eberwine, University of Pennsylvania Medical School, Philadelphia
T. Hughes, Montana State University, Bozeman
C. Lai, Scripps Research Institute, La Jolla, California
D. Lavery, Purdue Pharma LP, Cranbury, New Jersey

ASSISTANTS
L. Barrett, University of Pennsylvania Medical Center, Philadelphia
H. Butler, Montana State University, Bozeman
A. Dowell, The Scripps Research Institute, La Jolla, California
K. Haas, University of British Columbia, Vancouver, Canada
R. Lansford, California Institute of Technology, Pasadena
J. Tan, The Scripps Research Institute, La Jolla, California

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 served to illustrate 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 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 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 RT-PCR analyses from small numbers of cells (RNA purification, PCR optimization, interpretation of results); single-cell PCR and cDNA library construction; 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 that are available to molecular neuroscientists. This year's invited speakers included Don Baldwin (University of Pennsylvania), Catherine Berlot (Geisinger Clinic), Robert Darnell (HHMI/The Rockefeller University), Ardem Patapoutian (The Scripps Research Institute), Anne Marie Quinn (Montana Molecular, LLC), Heidi Scrable (University of Virginia), Lino Tessarollo (National Cancer Institute), and Frederick X. William Yang (David Geffen School of Medicine, UCLA).

PARTICIPANTS

Agathocleous, M., B.A., Cambridge University, United Kingdom
Brown, A., B.S., Stanford University, California
Chevere, I., B.S., University of Puerto Rico, San Juan
Drapeau, E., Ph.D., Columbia University, New York
Emsley, J., Ph.D., Harvard Medical School/Massachusetts General Hospital, Boston
Haag, N., Dipl., University Hospital Jena, Germany
Hammond, R., B.S./B.A., Oregon Health and Science University, Portland
Huberman, A., Ph.D., Stanford University, California
MacLeod, K., Ph.D., University of Maryland, College Park
Makino, Y., M.A., Johns Hopkins University School of Medicine, Baltimore, Maryland
Pellissier, F., M.S., University of Geneva, Switzerland
Perez, J., B.A., University of Michigan, Ann Arbor
Ryabinin, A., Ph.D., Oregon Health & Science University, Portland
Sands, T., B.A./B.S., Columbia University, New York
Veals, S., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Wilbrecht, L., Ph.D., Cold Spring Harbor Laboratory

SEMINARS

Berlot, C., Weis Center for Research, Danville, Pennsylvania: Splitting GFP to look at G-protein signaling.
Darnell, R., HHMI/The Rockefeller University, New York: Applying molecular biology to study the role of RNA-binding proteins in neurologic disease.
Eberwine, J., University of Pennsylvania Medical Center, Philadelphia: Molecular analysis of the neuronal dendrite: More and more surprises... .
Patapoutian, A., The Scripps Research Institute, La Jolla, California: How do you feel? Molecular basis of temperature sensation.
Scrable, H., University of Virginia, Charlottesville: Inducible expression in the mouse with the *lac* operator/repressor system.
Tessarollo, L., National Cancer Institute, Frederick, Maryland: Dissecting neurotrophin functions in vivo: Lessons from engineered mouse models.
Yang, X.W., University of California, Los Angeles: Mouse molecular genetic study of basal ganglia function and dysfunction.

Proteomics

July 8–21

INSTRUCTORS	**P. Andrews,** University of Michigan Medical School, Ann Arbor **J. La Baer,** Harvard Institute of Proteomics, Cambridge, Massachusetts **A. Link,** Vanderbilt University School of Medicine, Nashville, Tennessee
ASSISTANTS	**E. Hainsworth**, Harvard Medical School, Boston, Massachusetts **J. Jennings,** Vanderbilt University School of Medicine, Nashville, Tennessee **W. Montor,** University of Sao Paulo, Brazil **N. Ramachandran,** Harvard Institute of Proteomics, Cambridge, Massachusetts **E. Simon,** University of Michigan, Ann Arbor **S. Volk,** University of Michigan, Ann Arbor

This intensive laboratory and lecture course focused on two major themes in proteomics: protein profiling and functional proteomics. In the profiling section of the course, students learned about cutting-edge protein separation methods, including hand-on experience with two-dimensional gel electrophoresis, multidimensional liquid chromatography, and affinity purification of protein complexes. The course covered both MALDI and ESI high-sensitivity mass spectrometry including peptide mass map-

ping and tandem mass spectrometry, quantification using isobaric tags, and phosphoproteomics. Students learned to use several informatics tools available for analyzing the data. In the functional proteomics section of the course, students learned about recombinational cloning, high-throughput protein isolation, and protein microarrays. Students used robots to execute high-throughput methods including expression, purification, and characterization of proteins. They also printed and analyzed their own self-assembling protein microarrays, which were used for protein-protein interaction studies. The overall aim of the course was to provide each student 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 his/her own research. This year's speakers included Steven Carr (Broad Institute of Harvard and MIT), Brian Chait (The Rockefeller University), Pierre Chaurand (Vanderbilt University School of Medicine), Kevin Coombes (M.D. Anderson Cancer Center), Wade Hines (Beyond Genomics), Peter Juhasz (BG Medicine, Inc.), Akhilesh Pandey (Johns Hopkins University), Darryl Pappin (Applied Biosystems), Michael Snyder (Yale University), and Forest White (MIT).

PARTICIPANTS

Auger-Messler, M., Ph.D., Cincinnati Children's Hospital Medical Center, Cincinnati
Borhan, M., Ph.D., Saskatoon Research Centre, Canada
Callegari, E., Ph.D., University of South Dakota School of Medicine,Vermillion
Chung, J., Ph.D., McGill University, Montreal, Canada
Cora, E., Ph.D., University of Puerto Rico–Medical Sciences, San Juan
Esashi, F., Ph.D., Cancer Research, United Kingdom, Potters Bar, Herts
Gould, M., Ph.D., Universidad Autonoma de Baja California, Ensenada, B.C., Mexico
Huang, S., Ph.D., Baylor College of Medicine, Houston, Texas
Keech, O., M.S., Umea University, Sweden
Navarrete, M., B.S., Universidad Andres Bello, Santiago, Chile
Rossetti, S., Ph.D., Roswell Park Cancer Institute, Buffalo, New York
Scott, A., B.A., University of California, Berkeley
Soh, H., Ph.D., University of California, Santa Barbara
Valliyodan, B., Ph.D., University of Missouri, Columbia
Wood, N., Ph.D., GE Global Research, Niskayuna, New York
Ye, Y., Ph.D., Centers for Disease Control and Prevention, Atlanta, Georgia

SEMINARS

Carr, S., Broad Institute, Cambridge, Massachusetts: Biological mass spectrometry applications.
Chait, B., The Rockefeller University, New York: Hypothesis-driven mass spectrometry.
Chaurand, P., Vanderbilt University, Nashville, Tennessee: Imaging tissue by mass spectrometry.
Hines, W., Beyond Genomics, Cambridge, Massachusetts: Interpretating the tandem mass spectrum.
Juhasz, P., Beyond Genomics, Cambridge, Massachusetts: Biomaker discovery.
Pandey, A., Johns Hopkins University, Baltimore, Maryland: Protemics databases and signaling pathways.
Pappin, D., Applied Biosystems, Foster City, California: Quantitative mass spectrometry using isobaric tags.
Snyder, M., Yale University, New Haven, Connecticut: Protein arrays.
White, F., Massachusetts Institute of Technology, Cambridge: Phosphoproteomics.

Biology of Memory

July 22–August 4

INSTRUCTORS **K. Martin,** University of California, Los Angeles
J. Raymond, Stanford University, California

This lecture course provided an introduction to cellular, molecular, and systems approaches to learning and memory. It was suited for graduate students and postdoctoral fellows in molecular biology, neurobiology, and psychology, as well as research workers who are interested in an introduction to this field. The course covered topics ranging from behavioral considerations of learning and memory to gene regulation in the nervous system. The lectures provided an intensive coverage of modern behavioral studies of learning and memory, the cell and molecular biology of neuronal plasticity, cellular and molecular mechanisms of simple forms of learning and memory, and systems approaches to learning

in vertebrates and humans. Lectures were complemented by exercises in which students worked in small groups with lecturers to discuss topical issues in learning and memory, to evaluate recent studies, and to identify and formulate new research questions and approaches. The course is thus designed not only to introduce students to the field of learning and memory, but also to provide an intellectual framework upon which future studies can be built. Lecturers for this year's course include Jack Byrne, Yang Dan, Ron Davis, Howard Eichenbaum, Peter Holland, Mary Kennedy, Dan Madison, Earl Miller, Karim Nader, Larry Squire, Mriganka Sur, and Karel Svoboda.

The course was held at the Laboratory's Banbury Conference Center and was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Banerjee, S., Ph.D., Yale University, New Haven, Connecticut
Brasier, D., B.A., University of California, San Diego
Christensson, M., M.S., Lund University, Sweden
Einarsson, E., B.A., McGill University, Montreal, Canada
Jirenhed, D.-A., M.S., Lund University, Sweden
Kan, K.-H.C., M.S., Chinese University of Hong Kong
Ledderose, J., M.D., Universitat Freiburg, Germany
Lin, S.-C., M.D., Duke University Medical Center, Durham, North Carolina
Maczko, K., B.A., Stanford University, Menlo Park, California
Markram, K., M.A., Brain and Mind Intsitute, Lausanne, Switzerland
McKay, B., M.S., University of Calgary, Canada
Neunuebel, J., M.S., University of Texas Graduate School of Biomedical, Houston
Sakamoto, T., Ph.D., Okinawa Institute of Science and Technology, Uruma, Japan
Valente, A., M.A., Harvard University, Cambridge, Massachusetts
Wang, D., Ph.D., University of California, Los Angeles
Zhao, G., Ph.D., Stanford University, California

SEMINARS

Byrne, J., University of Texas/Houston Medical School: Learning mechanisms in aplysia.
Dan, Y., University of California, Berkeley: Spike-timing-dependent plasticity.
Davis, R., Baylor College of Medicine, Houston: Genetic approaches to learning and memory.
Eichenbaum, H., Boston University, Massachusetts: Role of the hippocampus.
Holland, P., Johns Hopkins University, Baltimore, Maryland: Psychology and ethology of learning; Learning theory.
Kennedy, M.. California Institute of Technology, Pasadena, California: Biochemistry of synaptic plasticity.
Madison, D., Stanford University, California: Synaptic plasticity: LTP and LTD.
Martin, K., University of California, Los Angeles: Cell biology of synaptic plasticity.
Miller, E., Massachusetts Institute Technology, Cambridge: The prefrontal cortex.
Nader, K., McGill University, Montreal, Canada: Consolidation and reconsolidation.
Raymond, J., Stanford University, California: Cerebellum-dependent learning.
Squire, L., University of California, San Diego: Memory systems in the brain; Medial temporal lobe.
Sur, M., Massachusetts Institute of Technology, Cambridge: Cortical plasticity: Networks.
Svoboda, K., Cold Spring Harbor Laboratory: In vivo imaging of cortical plasticity.

Eukaryotic Gene Expression

July 25–August 1

INSTRUCTORS	**L. Attardi,** Stanford University, California **M. Bulger,** University of Rochester School of Medicine, New York **A. Shilatifard,** St. Louis University, Missouri **L. Tora,** IGBMC, Illkirch, France
ASSISTANTS	**T. Hilton,** IGBMC, Illkirch, France **G. Isaacs,** Cornell University, Ithaca, New York **T. Johnson,** Stanford University, California **A. Wood,** St. Louis University Health Sciences Center, Missouri

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 both on 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. Characterizations of the DNA-binding properties of site-specific transcription factors were 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, including 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 RNAi 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. Speakers for 2005 were David Allis (The Rockefeller University), Genevieve Almouzni (Institut Curie), Shelley Berger (Wistar Institute), Keith Blackwell (Joslin Diabetes Center, Harvard Medical School), Stephen Buratowski (Harvard Medical School), David Bushnell (Stanford University), Joan Conaway (Stowers Institute for Medical Research), Sharon Dent (University of Texas, Anderson Cancer Center), Barbara Graves (University of Utah), Gordon Hager (National Cancer Institute), James Kadonaga (University of California, San Diego), Ramin Shiekhattar (The Wistar Institute), Jerry Workman (The Stowers Institute for Medical Research), and Carl Wu (National Cancer Institute, NIH).

PARTICIPANTS

Barrera, L., B.S., University of California, San Diego
Bolouri, H., Ph.D., Institute for Systems Biology, Seattle, Washington
Chandran, N., M.S., Wayne State University, Detroit, Michigan
Fager, V., B.S., Stockholm University, Sweden
Ferguson, P., Ph.D., University of South Carolina, Columbia
Fischer, J., M.S., Max-Planck Institute of Molecular Genetics, Berlin, Germany
Freitas, F., B.A., Instituto de Quimica, Sao Paulo State, Brazil
Gardini, A., M.S., The FIRC Institute of Molecular Oncology Foundation, Milan, Italy
Gordon, F., B.S., The Ohio State University Medical Center, Columbus
Maruggi, G., Ph.D., University of Modena, Italy
Mojzita, D., M.S., Gothenburg University, Sweden
Palsgaard, J., M.S., Hagedorn Research Institute & Copenhagen University, Gentofte, Denmark
Peiro, S., Ph.D., Institut d'Investigacio Medica, Bacelona, Spain
Segal, E., Ph.D., The Rockefeller University, New York
Sichero, L., Ph.D., Ludwig Institute for Cancer Research, Sao Paulo, Brazil
Walker, R., B.S., Meharry Medical College, Nashville, Tennessee

SEMINARS

Allis, D., The Rockefeller University, New York: Beyond the double helix: Writing and reading the histone code.
Almouzni, G., Institute Curie, Paris, France: Chromatin assembly from nucleosome to heterochromatin.
Attardi, L., Stanford University, California: Using mouse models to dissect the function of the p53 *trans*-activator in tumor suppression.
Berger, S., Wistar Institute, Philadelphia, Pennsylvania: Histone covalent modifications in gonomic regulation.
Blackwell, T.K., Harvard Medical School, Cambridge, Massachusetts: Transcription regulation in *C. elegans* oocytes and early embryos.
Bulger, M., University of Rochester, New York: Hyperacetylated domains and tissue-specific gene expression in erythroid cells.
Buratowski, S., Harvard Medical School, Cambridge, Massachusetts: Coupling transcription with chromatin and mRNA processing.
Bushnell, D., Stanford University, California: Structural insights into RNA polymerase II initiation and elongation.
Conaway, J., Stowers Institute for Medical Research, Kansas City, Missouri: Fundamental transcription mechanisms.
Dent, S., University of Texas, Houston: In vivo functions for histone-modifying enzymes.
Graves, B., University of Utah, Salt Lake City: ETS domain proteins: Signaling into the nucleus.
Hager, G., NCI/NIH, Bethesda, Maryland: The dynamics of transcription factor recruitment and promoter progression.
Kadonaga, J, University of California, San Diego: Studies of the RNA polymerase II core promoter.
Shiekhattar, R., The Wistar Institute, Philadelphia, Pennsylvania: Brave new world of small RNA.
Shilatifard, A., St. Louis University School of Medicine, Missouri: Yeast COMPASS points the way to human MLL and its role in pathogenesis of leukemia.
Tora, L., Institut de Genetique et de Biologie Moleculaire et Cellulaire, France: Different sets of promoter recognition factors regulate the transcription initiation of RNA polymerase II.
Workman, J., Stowers Institute for Medical Research, Kansas, Missouri: Protein complexes that activate or silence genes in chromatin.
Wu, C., NCI/NIH, Bethesda, Maryland: ATP-dependent chromatin remodeling complexes for transcription.

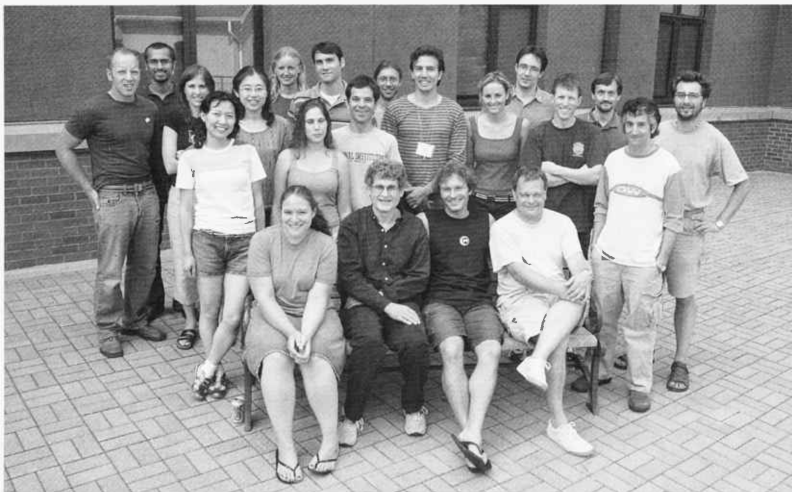
Imaging Structure and Function in the Nervous System

July 26–August 15

INSTRUCTORS
F. Engert, Harvard University, Cambridge, Massachusetts
M. Hubener, Max-Planck Institute of Neurobiology, Martinsried, Germany
D. Kleinfeld, University of California, San Diego

ASSISTANTS
A. Kampff, Harvard University, Cambridge, Massachusetts
V. Nagerl, Max-Planck Institute of Neurobiology, Martinsried, Germany
Q.T. Nguyen, University of California, San Diego
V. Staiger, Max-Planck Institute of Neurobiology, Martinsried, Germany
P. Tsai, University of California, San Diego
R. Vislay, Harvard University, Cambridge, Massachusetts
J. Waters, Northwestern University, Chicago, Illinois

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 intensive 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, functional 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, photoactivated ("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 and its variants. We used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells. Applicants had a strong background in the neurosciences or in cell biology. Lecturers in 2005 included William Betz (University of Colorado Medical School), Winfried Denk (Max-Planck Institute for Medical Research), Jahn Douglas (Coherent), Jason Eichenholz (Newport-Spectra Physics), Steven Fadul (University of Colorado Medical School), Oliver Griesbeck (Max-Planck Institute of Neurobiology), Stefan Hell (Max-Planck Institut für Biophysikalische Chemie), Mark Hobson (Hamamsutu), Fred Lanni (Carnegie-Mellon University), Jeff Lichtman (Harvard University), Jerome Mertz (Boston University), Valentin Nagerl (Max-Planck Institute of Neurobiology), James Remington (University of Oregon), Gordon Shepherd (Cold Spring Harbor Laboratory/HHMI), Karel Svoboda (HHMI, Cold Spring Harbor Laboratory), Sebastian Tille (Zeiss), Jack Waters (Northwestern University), Eiji Yokoi (Olympus American Inc.), and David Zenisek (Yale University).

This course was supported with funds provided by the National Institute of Mental Health, National Institute on Drug Abuse, and the Howard Hughes Medical Institute.

PARTICIPANTS

Aguirre, A., Ph.D., CNMC, Center for Neurosciences, Washington, D.C.
Cheng, L., B.S., The Johns Hopkins University School of Medicine, Baltimore, Maryland
Christie, J., Ph.D., Oregon Health & Sciences University, Portland
Gallio, M., Ph.D., University of California, San Diego
Hajj, G., Ph.D., Ludwig Intitute for Cancer Research, Sao Paulo, Brazil
Hsu, Y., Ph.D., University of Massachusetts, Amherst
Lang, S., M.A., Max-Planck Institute of Neurobiology, Martinsried, Germany
Pouille, F., Ph.D., University of California, San Diego
Rozsa, B., M.D., Institute of Experimental Medicine, Pharmacology, Budapest, Hungary
Sumbre, G., Ph.D., University of California, Berkeley
Uzzell, V., B.A., University of California, San Diego
van Welie, I., Ph.D., The Salk Institute for Biological Studies, La Jolla, California

SEMINARS

Betz, W., University of Colorado Medical School, Denver: Imaging secretion using FM 1-43.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: Frontiers in optics; Frontiers in EM.
Eichenholz, J., Newport-Spectra Physics: Theory of lasers.
Engert, F., Harvard University, Cambridge, Massachusetts: Introduction to tadpole preparation; Applications of tadpole preparations.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Germany: XFP-based Ca probes.
Hell, S., Max-Planck Institut für Biophysikalische Chemie, Gottingen, Germany: Nonlinear absorption and diffraction limits.
Hobson, M., Hamamastu: Principles and technology of CCD imaging.
Hubener, M., Max-Planck Institute of Neurobiology, Martinsried, Germany: Primer on intrinsic optical signals; Frequent domain methods for intrinsic signals.
Jahn, D., Coherent: Ultrafast lasers; OPAs, regenerative amps.
Kilborn, K., Intelligent Imaging Innovations, Inc., Santa Monica, California: Software for microscopy.
Kleinfeld, D., University of California, San Diego: Basic optical design; Basics of organic Ca indicators; Data analysis: Primer on the frequency domain; Data analysis: Noise sources and optimal S/N; Data analysis: Applications to imaging.
Lampff, A., Harvard University, Cambridge, Massachusetts: Introduction to image J.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Point spread functions; Diffraction theory; Confocal microscopy and grating imager.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Applications of confocal microscopy; Brainbow multilabels: Data analysis.
Mertz, J., Boston University, Massachusetts: Nonlinear techniques.
Nagerl, V., Max-Planck Institute of Neurobiology, Munich, Germany: Applications of 2p imaging.
Nguyen, Q.T., University of California, San Diego: Brain, brainstem, and semi-intact preparations.
Remington, J., University of Oregon, Eugene: The world of XFPs.
Shepherd, G., Cold Spring Harbor Laboratory: Caged compounds and circuit analysis.
Svoboda, K., HHMI, Cold Spring Harbor Laboratory: Basics of 2p imaging; Advanced applications of 2p microscopy; FRET and fluorescence lifetime imaging.
Tille, S., Zeiss: Overview of the Zeiss Meta and Pascal.
Tsai, P., University of California, San Diego: Fluorescence.
Waters, J., Northwestern University, Chicago, Illinois: Advanced aspects (loading, quantitative modeling) of organic Ca indicators.
Yokoi, E., Olympus American Inc., Melville, New York: Overview of Olympus FluoView.
Zenisek, D., State University of New York, Stony Brook: Total internal reflectance fluorescence (TIRF).

Yeast Genetics and Genomics

July 26–August 15

INSTRUCTORS

D. Amberg, State University of New York, Syracuse
D. Burke, University of Virginia, Charlottesville
J. Strathern, National Cancer Institute, Frederick, Maryland
M. Whiteway, NRC Biotechnology Research Institute, Montreal, Canada

ASSISTANTS

S. Li, SUNY Upstate Medical Center, Syracuse, New York
M. Sambade, University of Texas Southwestern Medical Center, Dallas
D. Waller, NRC Biotechnology Research Institute, Montreal, Canada

This 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 were 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 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 interactions, including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpreted experiments with DNA

arrays. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, 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 Brenda Andrews (University of Toronto), Kerry Bloom (University of North Carolina, Chapel Hill), Jeff Boeke (Johns Hopkins University), Marian Carlson (Columbia University), Kara Dolinski (Princeton University), Beverly Errede (University of North Carolina, Chapel Hill), David Garfinkel (NCI-FCRDC ABL-Basic Research Program), Frank Luca (University of Pennsylvania), Aaron Mitchell (Columbia University), Jasper Rine (University of California, Berkeley), George Sprague (University of Oregon), Kevin Struhl (Harvard Medical School), and Reed Wickner (National Institutes of Health).

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Cole, S., B.S., Brandeis University, Waltham, Massachusetts
Fadri, M.T., B.S., Baylor College of Medicine, Houston, Texas
Hessa, T., B.S., Stockholm University, Sweden
Hutchins, G., Ph.D., Fort Lewis College, Durango, Colorado
Li, H., Ph.D., University of California, San Francisco
Lindroos, H., M.S., Karolinska Institute, Stockholm, Sweden
Locke, J., M.S., University of Warwick, Coventry, United Kingdom
Morris, G., B.S., University of Chicago, Illinois
Narayan, S., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Ocampo, M.T., B.S., New York University
Perocchi, F., Laurea., EMBL, Heidelberg, Germany
Ramachandran, G., B.S., Massachusetts Institute of Technology, Cambridge
Segal, E., Ph.D., The Rockefeller University, New York
Smith, A., B.A., Northwestern University, Evanston, Illinois
Smith, V., Ph.D., Duke University Medical Center, Durham, North Carolina
Upadhyaya, A., Ph.D., Massachusetts Institute of Technology, Cambridge

SEMINARS

Andrews, B., University of Toronto, Canada: An embarrassment of riches: Yeast genetics, functional genomics, and the cell cycle.
Bloom, K., University of North Carolina, Chapel Hill: Quantitative microscopy and molecular modeling to dissect mechanisms of chromosome segregation.
Boeke, J., Johns Hopkins University School of Medicine, Baltimore, Maryland: Functional genomics of the yeast genome integrity network.
Burke, D., University of Virginia Medical Center, Charlottesville: The spindle checkpoint: Orchestrating the mitotic minuet.
Carlson, M., Columbia University, New York: Snfl/AMPK pathway and nutrient signals in yeast.
Dolinski, K., Princeton University, New Jersey: Introduction to the *Saccharomyces* genome database.
Errede, B., University of North Carolina, Chapel Hill: MAPK pathways controlling mating differentiation and pseudohyphal development in *S. cerevisae*.
Garfinkel, D., National Cancer Institute, Bethesda, Maryland: How budding yeast and Ty retrotransposons coexist.
Luca, F., University of Pennsylvania, Philadelphia: Yeast MEN and RAM: Two signaling networks that control mitotic exit, morphogenesis, and daughter-specific transcription.
Mitchell, A., Columbia University, New York: Extending APOYG into the pathogen *Candida albicans*.
Sprague, G., University of Oregon, Eugene: Control of cell type and signal transduction specificity.
Struhl, K., Harvard Medical School, Cambridge, Massachusetts: Transcriptional regulatory mechanisms.
Wickner, R., National Institutes of Health, Bethesda, Maryland: Yeast prions: Proteins can be genes.

C. elegans

July 28–August 15

INSTRUCTORS
S. Clark, New York University School of Medicine
M. de Bono, MRC/Cambridge, United Kingdom
M. Labouesse, CNRS/INSERM/UL, France

ASSISTANTS
L. Moffat, New York University School of Medicine
A.-S. Nicot, IGBMC/CNRS/INSERM, France
B. Olofsson, MRC/Cambridge, United Kingdom

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 have 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 picking, *C. elegans* data-

bases 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 them to embark on their own research projects after returning to their home institutions. Speakers in the 2005 course included Jean-Louis Bessereau (Ecole Normale Superieure–INSERM), Mark Blaxter (University of Edinburgh), Craig Hunter (Harvard University), Chris Li (City College of New York), Rueyling Lin (UTSW Medical Center), Kiyoji Nishiwaki (RIKEN Center for Developmental Biology), Amy Pasquinelli (University of California, San Diego), Fabio Piano (New York University), Joel Rothman (University of California, Santa Barbara), Jonathan Scholey (University of California, Davis), Lincoln Stein (Cold Spring Harbor Laboratory), and Kevin Strange (Vanderbilt University Medical Center).

This course was supported with funds provided by the National Institute of Child Health and Human Development.

PARTICIPANTS:

Choe, K., Ph.D., Vanderbilt University, Nashville, Tennessee
Cole, S., Ph.D., University of Virginia, Charlottesville
Dolgin, E., B.S., University of Edinburgh, United Kingdom
Fleming, A., Ph.D., Syngenta, Bracknell, Berkshire, United Kingdom
Hayward, S., Ph.D., University of Liverpool, United Kingdom
Hicks, E., B.S., ViaLactia Biosciences, Auckland, New Zealand
Hobson, R., B.S., University of Toledo, Ohio
Horn, V., Ph.D., Lausanne University, Switzerland
Jaedicke, A., Ph.D., EMBL, Heidelberg, Germany
Kato, M., Ph.D., National Institute of Genetics, Mishima, Japan
Reid, S., Ph.D., University of Auckland, New Zealand
Saha, S., Ph.D., Boston University, Massachusetts
Sauvage, P., B.S., Université Paris, France

SEMINARS

Bessereau, J.-L., Inserm, Ecole Normale Superieure, Paris, France: Building synapses in *C. elegans:* From ultrastructure to gene function.
Blaxter, M., University of Edinburgh, United Kingdom: Comparing nematode genomes across billions of years of evolution.
Clark, S., New York University School of Medicine: Establishment of neuronal polarity.
de Bono, M., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: Oxygen sensing and the evolution of foraging behavior in *C. elegans*.
Hunter, C., Harvard University, Cambridge, Massachusetts: Molecular genetics of systemic RNAi.
Labouesse, M., CNRS/IGBMC, Strasbourg, Germany: Squeezing a ball into a tube: From genes to possible forces.
Li, C., City College of New York: Using *C. elegans* as a model for Alzheimer's disease.
Lin, R., University of Texas Southwestern Medical Center, Dallas: Cell fate specification in early embryos.
Nishiwaki, K., RIKEN Center for Developmental Biology, Kobe, Japan: Genetic analysis of gonad morphogenesis.
Pasquinelli, A., University of California, San Diego: MicroRNAs: A small contribution from worms.
Piano, F., New York University: From genotype to phenotype: Molecular models of early embryogenesis in *C. elegans*.
Rothman, J., University of California, Santa Barbara: Biological switches: Life, growth, and death in *C. elegans*.
Scholey, J., University of California, Davis: Intraflagellar transport *C. elegans* neurons: From molecular motors to ciliary disease.
Stein, L., Cold Spring Harbor Laboratory: Wormbase.
Strange, K., Vanderbilt University Medical Center, Nashville, Tennessee: Integrative physiology of salt and water homeostatsis: New insights from an old worm.

Cellular Biology of Addiction

August 9–15

INSTRUCTORS **B. Madras,** Harvard Medical School, Southborough, Massachusetts
M. von Zastrow, University of California, San Francisco

ASSISTANT **A. Jassen,** Harvard Medical School, Southborough, Massachusetts

Drug addiction is the most costly neuropsychiatric disorder faced by our nation. Acute and repeated exposure to drugs produces neuroadaption and long-term memory of the experience, but the cellular and molecular processes involved are only partially understood. The primary objective of the workshop was to provide an intense dialog 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, withdrawal, craving, and relapse to drug use and to brain function, in general.

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 cellular level, neural networks and their modulation; the relevance of genotype to susceptibility and drug response; tolerance and adaptation at the cellular level, and approaches to exploiting the daunting volume generated by neuroinformatics. This workshop provided an integrated view of current and novel research on neuroadaptive responses to addiction, 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 on the neurobiological consequences of drug use and addiction. The workshop was designed to generate interest in this level of analysis, open conduits for collaborations, and present novel routes to investigating the neurobiology of addictive drugs.

Speakers in the 2005 course included George Augustine (Duke University Medical Center), Randy Blakely (Vanderbilt University School of Medicine), Teresa Branchek (Lundbeck Research America), Emery Brown (Massachusetts General Hospital), Marc Caron (Duke University Medical Center), Robert Edwards (University of California, San Francisco), Chris Evans (University of California, Los Angeles), Gordon Fishell (The Skirball Institute, NYU Medical Center), Joel Gelernter (Yale University School of Medicine), David Goldman (NIAAA/LNG), Peter Kalivas (Medical University of South Carolina), Mary Jean Kreek (The Rockefeller University), Angus Nairn (Yale University School of Medicine), Eric Nestler (University of Texas Southwestern Medical Center), Daniele Piomelli (University of California, Irvine), Michael Rosenfeld (University of California, San Diego), George Uhl (NIDA), and Renping Zhou (Rutgers University College of Pharmacy).

The course was held at the Laboratory's Banbury Conference Center and was supported with funds provided by the National Institute of Drug Abuse.

PARTICIPANTS

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Bowers, M.S., Ph.D., University of California, San Francisco
Corbit, L., Ph.D., Ernest Gallo Clinic and Research Center, Emeryville, California
Graber, S., Ph.D., The Burnham Institute, La Jolla, California
Heifets, B., B.S., Albert Einstein College of Medicine, Bronx, New York
Hubert, G., Ph.D., Emory University, Atlanta, Georgia
Jacobs, M., B.A., Vanderbilt University Medical Center, Nashville, Tennessee
Jung, H.Y., Ph.D., HHMI/Massachusetts Institute of Technology, Cambridge
Kash, T., B.S., Vanderbilt University Medical Center, Nashville, Tennessee
Lawrence, D., Ph.D., National Institute on Drug Abuse, Bethesda, Maryland
Lin, Z., Ph.D., Harvard Medical School, Southborough, Massachusetts
Mangieri, R., B.A., University of California, Irvine
Melendez, R., Ph.D., Medical University of South Carolina, Charleston
Perrine, S., Ph.D., Temple University School of Medicine, Philadelphia, Pennsylvania
Simmons, D., B.S., University of Texas Southwestern Medical Center, Dallas
Sipe, J., M.D., The Scripps Research Institute, La Jolla, California
Stowers, S., Ph.D., University of California, Berkeley
Tietz, E., Ph.D., Medical College of Ohio, Toledo
Valdez, G., Ph.D., Harvard Medical School, Southborough, Massachusetts
Wang, H., Ph.D., University of Toronto, Canada
Wang, J.C., Ph.D., Washington University, St. Louis, Missouri
Wolfe, J., Ph.D., University of Pennsylvania, Philadelphia
Zamanian, J., Ph.D., Stanford University, California

Augustine, G., Duke University Medical Center, Durham, North Carolina: Synapsins: Roles in synaptic vesicle trafficking and behavioral responses to psychotropic drugs.

Biederer, T., Yale University, New Haven, Connecticut: Mechanisms of synaptic differentiation.

Branchek, T., Lundbeck Research, Paramus, New Jersey: Trace amines and their receptors.

Breiter, H., Massachusetts General Hospital, Boston: Brain mapping and the circuitry of reward/aversion.

Brown, E., Massachusetts General Hospital, Boston: Dynamic analyses of neural representations using the state-space modeling paradigm.

Caron, M., Duke University Medical Center, Durham, North Carolina: Using animal models to probe the neuronal plasticity and signaling associated with drugs of abuse.

Evans, C., University of California, Los Angeles: Endogenous and exogenous opioids in pain and leasure.

Fishell, G., New York University Medical Center: Developmental origins of cortical interneurons and the basal ganglia.

Galli, A., Vanderbilt University, Nashville, Tennessee: Molecular biophysics of amphetamine action.

Gasic, G., Massachusetts General Hospital, Boston: Neural system bases of addictive behaviors.

Gelernter, J., Yale University School of Medicine, New Haven, Connecticut: Genetic linkage studies of drug dependence and related phenotypes: Brief outline.

Goldman, D., NIAAA, Rockville, Maryland

Kalivas, P., Medical University of South Carolina, Charleston: Neuroplasticity as a pharmacotherapeutic target in addiction. Can we forget to be addicted?

Kreek, M.J., The Rockefeller University, New York: Endorphins, gene polymorphisms, stress responsivity, and specific addictions: Selected topics.

Nestler, E., University of Texas Southwestern Medical Center, Dallas: Transcriptional mechanisms underlying drug addiction.

Piomelli, D., University of California, Irvine: The endogenous cannabinoid system and the treatment of drug abuse.

Pollack, J., NIDA/National Institutes of Health, Bethesda, Maryland: The funding process at NIH.

Rosenfeld, M., University of California, San Diego: Sensors and signals: Integration of transcriptional response in development and disease.

Tonegawa, S., RIKEN-MIT Neuroscience Research Center, Cambridge, Massachusetts: Complex genetics of alcoholism and other addictions.

Uhl, G., NIDA-IRP, National Institutes of Health, Baltimore, Maryland: Molecular genetics of addiction.

von Zastrow, M., University of California, San Francisco: Using receptor cell biology as a window into opiate drug action and addiction.

Zhou, R., Rutgers University, Piscataway, New Jersey: Specification of the midbrain dopaminergic pathways.

Workshop on Cereal Genomics

September 13–19

INSTRUCTORS	**S. Hake**, USDA/University of California, Berkeley Plant Gene Expression Center **D. Jackson**, Cold Spring Harbor Laboratory **L. Stein**, Cold Spring Harbor Laboratory **D. Ware**, Cold Spring Harbor Laboratory
ASSISTANTS	**P. Bommert**, Cold Spring Harbor Laboratory **W. Zhao**, Cold Spring Harbor Laboratory

This 1-week workshop enabled participants to take advantage of emerging genetic tools and genomics data in the cereals, including the complete genome sequence of rice and extensive EST and genome survey sequence from maize, wheat, and other grasses. The workshop featured morning and evening lectures with afternoon computer lab exercises. The workshop also provided hands-on lab work in the comparative anatomy, phenotype, and QTL sections in which participants examined samples and made measurements for statistical analysis to illustrate the power of maize genetics and its relation to genomics. The faculty (instructors and lecturers) were active researchers in cereal genetics and

genomics who have made significant contributions to the field, ensuring that the latest techniques and ideas were presented. The course was structured to provide time for informal discussions and exchanges with leaders in the field. Topics included the rice genome and its annotation; genetic mapping; linking physical and genetic maps; classical maize genetics and tools; comparative anatomy of the cereals; comparative genomics; the maize genome organization and "partial" sequence; cereals databases; quantitative trait locus mapping, association analysis and evolution; and genome-wide expression analysis: ESTs, microarrays, and MPSS. Speakers in the workshop included Jeff Bennetzen (University of Georgia, Athens), Ed Buckler (USDS-ARS Cornell University), Robin Buell (TIGR, The Institute for Genomic Research), Katrien Devos (University of Georgia, Athens), Elizabeth Kellogg (University of Missouri, St. Louis), Lisa Harper (University of California, Berkeley), Claire Hebbard (Gramene), Carolyn Lawrence (USDA-ARS, Iowa State University), Robert Martienssen (Cold Spring Harbor Laboratory), Blake Meyers (Delaware Biotechnology Institute), Torbert Rocheford (University of Illinois, Urbana), Carl Schmidt (University of Delaware), Robert Schmidt (University of San Diego), Scott Tingey (Dupont Company), Virginia Walbot (Stanford University), and Cliff Weil (Purdue University).

This workshop was supported with funds provided by the National Science Foundation.

PARTICIPANTS

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Engle, J., Ph.D., USDA-ARS, Raleigh, North Carolina
Goicoechea, J.L., B.S., Arizona Genomics Institute, Tuscon,
Held, M., Ph.D., Purdue University, West Lafayette, Indiana
Hunter, C., B.S., University of Florida, Gainesville
McGill, M.A., B.S., University of Wisconsin, Madison
Okori, P., Ph.D., Makerere University Uganda, Uppsala, Sweden
Paszkowski, U., Ph.D., University of Geneva, Switzerland
Persson, M., B.S., Swedish University of Agricultural Sciences, Uppsala, Sweden
Preston, J., Ph.D., University of Missouri, St. Louis
Reddy, L., B.S., Washington State University, Pullman
Sakai, H., B.S., National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan
Stonaker, J., B.S., University of California, Berkeley
Zhang, X., Ph.D., University of Georgia, Athens

SEMINARS

Bennetzen, J., Ph.D., University of Georgia, Athens: Maize genome organization and sequencing strategies.
Buell, C.R., The Institute for Genomic Research, Rockville, Maryland: Strategies to sequence and annotate the "complete" rice genome.
Devos, K., Ph.D., University of Georgia, Athens: Comparative cereal genomics.
Harper, L., University of California, Berkeley: Meiosis, recombination, and mapping.
Kellogg, E., University of Missouri, St. Louis/Schmidt, R., University of San Diego, California/Hake, S., Plant Gene Expression Center, USDA, Albany, California: Introduction to the cereals: Comparative anatomy and mutants.
Martienssen, R., Cold Spring Harbor Laboratory: Maize transposons, genomics, and epigenomics.
Rocheford, T., Ph.D., University of Illinois, Urbana/Buckler, E., North Carolina State University, Raleigh: Quantitative trait locus mapping, Association analysis and diversity.
Tingey, S., Dupont Company, Newark, Delaware: Future directions in cereal genomics.
Walbot, V., Stanford University, California/Meyers, B., Delaware Biotechnology Institute, Newark, Delaware: ESTs, gene models, and microarrays: MPSS and SAGE.
Weil, C., Purdue University, West Lafayette, Indiana: Reverse genetics.

X-ray Methods in Structural Biology

October 10–25

INSTRUCTORS **W. Furey,** V.A. Medical Center, Pittsburgh, Pennsylvania
G. Gilliland, Centorcor, Inc., Radnor, Pennsylvania
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku/MSC, Inc., The Woodlands, Texas

ASSISTANT **N. Tolia,** Cold Spring Harbor Laboratory

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 crystallogra-

phy. 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, molecular graphics, structure refinement, structure validation, coordinate deposition, and structure presentation. Participants learned through extensive hands-on experiments. One or more proteins were crystallized, and the structure(s) was determined by several methods, 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 Linux workstations using a screen-based editor (either vi or emacs).

PARTICIPANTS

Calabrease, M., B.S., Yale University, New Haven, Connecticut
Chen, L., Ph.D., Henry M. Jackson Foundation, Bethesda, Maryland
Chitnumsub, P., Ph.D., National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand
De Mattheis, C., Ph.D., The University of Notthingham, United Kingdom
Gordon, W., Ph.D., Harvard Medical School/Brigham & Women's Hospital, Boston, Massachusetts
Grundner, C., B.S., University of California, Berkeley
Henriksson, L., M.S., Uppsala University, Sweden
Hierro, A., Ph.D., NIH/NIDDK, Bethesda, Maryland
Kuhn, C., M.S., University of Munich, Germany
Matei, E., Ph.D., NIH/NIDDK, Bethesda, Maryland
Park, S., Ph.D., Inha University, Incheon, Massachusetts
Sayre, R., Ph.D., Ohio State University, Columbus, Ohio
Slotboom, D., Ph.D., University of Groningen, Netherlands
Sobolevsky, A., Ph.D., Columbia University, New York
Ulens, C., Ph.D., Netherlands Cancer Institute, Amsterdam, Netherlands
Xu, M., M.S., Novartis, Cambridge, Massachusetts

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, Berkeley, California: Introduction to CNS and PHENIX; Structure refinement.
Dauter, Z., Brookhaven National Laboratory, Upton, New York: Anomalous data collection.
Furey, W., V.A. Medical Center, Pittsburgh, Pennsylvania: Patterson group therapy; Isomorphous replacement and anomalous scattering; Scaling and merging synchrotron data; MAD phasing: A classical approach; Solvent flattening/phase combination; Solving structures with BnP; Noncrystallographic symmetry averaging.
Gilliland, G., Centorcor Inc., Radnor, Pennsylvania: Crystallization databases and strategies.
Hendrickson, W., Columbia University, New York: MAD phasing: Theory and practice.
Hung, Li-Wei, Los Alamos National Laboratory, Berkeley, California: SOLVE and RESOLVE; Just because it's in *Nature*, doesn't mean it's true...(macromolecular structure validation).
Joshua-Tor, L., Cold Spring Harbor Laboratory: Structure presentation; Argonaute: The secret of Slicer.
Kjeldgaard, M., Aarhus University, Denmark: Electron density fitting from A to 0.
McPherson, A., University of California, Irvine: Crystallization of macromolecules I; Crystallization of macromolecules II; Symmetry, periodicity, unit cells, space groups, miller planes, and lattices; Waves, vectors, and complex numbers; Fundamental diffraction relationships and Braggs law; Diffraction patterns, reciprocal space, and Ewald's sphere; Heavy atoms and anomalous scatterers; Fourier transforms and the electron density equation; Pattern techniques; Mechanisms of crystal growth.
Perrakis, A., Netherlands Cancer Institute, Amsterdam: Automated model building and refinement with ARP/WARP; Shaping the human genome: Structural insights on the action of retrotransposons.
Pflugrath, J., Rigaku/MSC, Inc., The Woodlands, Texas: Data collection: Design and setup I; Data collection: Design and setup II; Cryocrystallography; Away from the Edge: Sulfur SAD with chromium radiation.
Read, R., University of Cambridge, United Kingdom: Molecular replacement: From Pattersons to likelihood; Likelihood: Ideas and applications.
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; Macromolecular refinement II; Difference electron density maps.
Waugh, David, National Cancer Institute, Frederick, Maryland: Maximum likelihood strategies for protein expression and purification.
Westbrook, J., Rutgers University, Piscataway, New Jersey: The protein data bank.
Xu, R.-M., Cold Spring Harbor Laboratory: Structural mechanisms of epigenetic inheritance.

Advanced Bioinformatics

October 12–25

INSTRUCTORS
S. Lewis, University of California, Berkeley
S. Prochnik, Berkeley *Drosophila* Genome Project, California
L. Stein, Cold Spring Harbor Laboratory
J. Tisdall, DuPont Corporation, Wilmington, Delaware

ASSISTANTS
J. Babayev, Pioneer Hi-Bred, Johnston, Iowa
S.J. Cain, Cold Spring Harbor Laboratory
D. Curiel, Pioneer Hi-Bred, Johnston, Iowa
J. Karalius, Seminis Vegetable Seeds, Inc., Woodland, California
T. Marques, Universitat Pompeu Fabra, Barcelona, Catalunya, Spain
S. Robb, University of Utah, Salt Lake City
L. Teytelman, Cold Spring Harbor Laboratory
C. Yamada, University of Cambridge, United Kingdom

Today, the computer is an indispensable part of a research biologist's toolkit. The success of the human and other organisms genome projects has created terabytes of data on everything from genetic linkage mapping, to nucleotide sequences, to protein structures, stashed away in databases around

the globe. Large-scale technologies such as DNA microarrays and high-throughput genotyping have transformed the nature of laboratory experimentation. Furthermore, even when biologists are not generating large data sets of their own, they will want to collect and analyze data from myriad sources in the pursuit of novel candidates or even entire research avenues. A few years ago, it might have been sufficient to use Excel spreadsheets for managing laboratory data and canned Web interfaces for searching, but as the volume of data grows and the subtlety of analysis increases, these techniques, even supplemented by some simple programming skills, have become inadequate. Modern biologists must be adept at juggling disparate data sets in order to pursue their research. Designed for students and researchers with some prior programming experience, the 2-week Advanced Bioinformatics program gave biologists the expanded bioinformatics skills necessary to construct computational systems that can exploit this increasingly complex information landscape, with an emphasis on fitting the wide range of existing analysis tools into extensible bioinformatics systems. The course combined formal lectures with hands-on sessions in which students worked to solve a series of problem sets covering common scenarios in the acquisition, validation, integration, analysis, and visualization of biological data.

For their final projects, students posed problems using their own data and worked with one another and the faculty to solve them. The prerequisites for the course was basic knowledge of UNIX, procedural Perl programming, HTML document creation, and the database query language, SQL. Lectures and problem sets covering this background material were available online and students could study this material before starting the course.

The primary focus of this course was to provide students with the practical aspects of software development, rather than to present a detailed description of the algorithms used in computational biology.

Speakers in the 2005 course included Emina Begovic (University of California, Berkeley), Peter Brokstein (DOE Joint Genome Institute), Roderic Guigo (Institut Municipal d'Investigacio Medica), Winston Hide (University Western Cape, South Africa), Garbor Marth (Boston College), Sheldon McKay (Cold Spring Harbor Laboratory), Chris Mungall (Berkeley *Drosophila* Genome Project), Lior Pachter (University of California, Berkeley), William Pearson (University of Virginia), Jason Stajich (Duke University), Paul Thomas (Applied Biosystems), and Olga Troyanskay (Princeton University).

PARTICIPANTS

Arziman, B.Z., M.S., German Cancer Research Center, Heidelberg
Aziz, R., B.S., University of Tennessee, Memphis
Berglund, L., M.S., Royal Institute of Technology, Stockholm, Sweden
Ding, Y., Ph.D., The Jackson Laboratory, Bar Harbor, Maine
Dirk, W., M.S., University of California, Berkeley
Dixon, J., Ph.D., Johnson and Johnson, Spring House, Pennsylvania
El Karoui, M., Ph.D., Institut National de la Recherche Agronomique Jouy en Josas, France
Gopinathrao, G., Ph.D., Cold Spring Harbor Laboratory
Heiser, L., Ph.D., Lawrence Berkeley National Laboratory, Berkeley, California
Hoffman, M., B.A., Boston College, Chestnut Hill, Massachusetts
Kao, C.-F., Ph.D., University of Mexico, Albuquerque, New Mexico
Kavanaugh, L., M.S., Duke University, Hillsborough, North Carolina
Manabe, Y., Ph.D., Agriculture and Agri-Food Canada, Ottawa
Messina, D., M.A., Washington University School of Medicine, St. Louis, Missouri
Mottagui, S., Ph.D., Karolinska Institutet, Stockholm, Sweden
Nayak, V., M.S., University of Pennsylvania, Philadelphia
Papadopoulos, C., B.S., University of Georgia, Athens
Rodriguez-Zapata, F., B.S., International Center for Tropical Agiculture, Cali, Valle, Colombia
Salih, H., B.S., Texas A&M University, College Station
Stillwell, R., B.S., University of Hawaii, Manoa, Honolulu

Begovic, E., University of California, Berkeley: Molecular evolution: Molecular clocks, ka/ks, 4DTV; Gene prediction: Training and running programs.

Guigo, R., Institut Municipal d'Investigacio Medica, Barcelona, Spain: Gene prediction in ENCODE.

Lewis, S., University of California, Berkeley: Biological ontologies: Organizing information in machine-readable form; Graphical visualization systems: Gbrowse, UCSC browser, Apollo, IGB.

Marth, G., Boston College, Chestnut Hill, Massachusetts: Sequence variation analysis: SNPs, haplotyes.

McKay, S., Cold Spring Harbor Laboratory: Querying SQL databases with DBI.pm.

Mungall, C., Berkeley *Drosophila* Genome Project, Berkeley, California: Biological database design; Designing and running bioinformatics pipelines with Perl.

Pachter, L., University of California, Berkeley: Aligning genomes: Global and local (*multiple) whole-genome alignment tools and considerations.

Pearson, W., University of Virginia, Charlottesville: Aligning proteins: Local/global alignment algorithms (Smith-Waterman and blastp, etc.) + substitution matrices, part 1; Aligning proteins: Local/global alignment algorithms (Smith-Waterman and blastp, etc.) + substitution matrices part 2; Problem set: Protein alignments.

Prochnik, S., Princeton University, New Jersey: Designing and writing object-oriented Perl: Writing your own Perl classes.

Stajich, J., Duke University, Durham, North Carolina: Introduction to Bioperl: Organization and the basic classes; Bioperl II: Using Bioperl: More classes and using them.

Stein, L., Cold Spring Harbor Laboratory: Perl review I: Files, system calls, basic IO, subroutines; Perl review II: Data structures, regular expressions; Using object-oriented programming in Perl: Examples of using off-the-shelf Perl modules; Creating web graphics in Perl: GD.pm.

Thomas, P., Applied Biosystems, Foster City, California: Functional assignment, protein domain assignment, COGs, panther, pfam.

Tisdall, J., DuPont Experimental Station, Wilmington, Delaware: Speeding it up: Using multiple processors and clusters to run complex jobs faster.

Troyanskaya, O., Princeton University, New Jersey: Introduction to large-scale expression studies: Microarrays.

Winning the plate race

Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 21–November 3

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

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 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 present up-to-the-minute reports on current methods and research using the techniques being presented.

Speakers in this year's course included Richard Day (University of Virginia), Mats Gustafsson (University of California, San Francisco), Alexey Khodjakov, Thomas Ried (NCI/NIH), David Spector (Cold Spring Harbor Laboratory), Phong Tran (University of Pennsylvania), and Lani Wu (UTSW).

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Beebe, D., Ph.D., University of Wisconsin, Madison
Beser, A., M.D., Istanbul University Oncology Institute, Turkey
Campbell, J., B.S., Washington University, St. Louis, Missouri
Carvalho, F., M.S., Cold Spring Harbor Laboratory
Colgan, L., B.S., Center for Neuroscience, University of Pittsburgh, Pennsylvania
Hinas, A., M.S., Swedish University of Agricultural Sciences, Uppsala, Sweden
Ivakhno, S., M.S., Institute of Molecular Biology and Genetics, Kyiv, Ukraine
Lampe, T., Dipl., University of Goettingen, Germany
Leung, A., Ph.D., Massachusetts Institute of Technology, Cambridge
Manwell, L., B.S., University of Waterloo, Canada
Martin, J., B.S., University of Florida, Gainesville
Orlando, E., Ph.D., Florida Atlantic University at Harbor Branch Oceanographic Institution, Ft. Pierce
Schmid, D., M.S., The Rockefeller University, New York
Viklund, I.-M., Ph.D., Karolinska Institutet, Stockholm, Sweden
Weber, L., Ph.D., University of Colorado, Boulder
Zeiss, C., Ph.D., Yale University, New Haven, Connecticut

SEMINARS

Day, R., University of Virginia, Charlottesville: Seeing colors: Applications and limitations of the fluorescent proteins.
Dernburg, A., Lawrence Berkeley National Laboratory, Berkeley, California: Basic of DNA FISH.
Gustafsson, M., University of California, San Francisco: Increased resolution fluorescence imaging by linear and nonlinear structured illumination microscopy.
Hu, K., Scripps Research Institute, La Jolla, California/Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Basic introduction to light and fluorescence microscopy.
Khodjakov, A., Wadsworth Center, Albany, New York: Studies on mitosis.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Immunocytochemistry.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia/Swedlow, J., University of Dundee, United Kingdom: Principles of confocal microscopy and deconvolution techniques.
Ried, T., NCI/NIH, 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.
Tran, P., University of Pennsylvania, Philadelphia: Cameras and digital imaging fundamentals.
Wu, L., University of Texas Southwestern Medical Center, Dallas: Cytological profiling.

Computational Genomics

November 2–8

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
R. Smith, GlaxoSmithKline, King of Prussia, Pennsylvania

ASSISTANT **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 and 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.

The course was designed for biologists seeking advanced training in biological sequence analysis, for computational biology core resource directors and staff, and for scientists in other disciplines, such as computer science, who wish to survey current research problems in biological sequence analysis. The primary focus was the theory and practice of algorithms used in computational biology, with the goal of using current methods more effectively and developing new algorithms.

Speakers in the 2005 course included Stephen Altschul (National Library of Medicine), Peter Cooper (NCBI/NLM), Peter D'Eustachio (New York University Medical Center), Ross Hardison (Penn State University), and Mark Yandell (HHMI/University of Utah).

PARTICIPANTS

Apostolou, S., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Brodie, S., B.S., State University of New York, Buffalo
Carvalho, F., M.S., Cold Spring Harbor Laboratory
Colombo, T., M.S, New York University
Crow-Sanchez, K., Ph.D., Yale University, New Haven, Connecticut
Kingan, S., B.S., Harvard University, Cambridge, Massachusetts
Krasnitz, A., Ph.D., Cold Spring Harbor Laboratory
MacConaill, L., Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts
Pishotta, F., M.A., Mayo Clinic, Jacksonville, Florida
Rosengarten, R., B.A., Yale University, New Haven, Connecticut
Segal, C., M.S., University of Mexico, Mexico City
Sugino, K., Ph.D., Brandeis University, Waltham, Massachusetts
Venkatraman, A., Ph.D., Texas A&M University, College Station
Wongratanacheewin, S., Ph.D., Khon Kaen University, Thailand
Zerlotini N.A., B.S., Rene Rachou Research Center–Oswaldo Cruz Foundation, MG, Brazil

SEMINARS

Altschul, S., National Library of Medicine, Bethesda, Maryland: Statistics of sequence similiarity scores; Iterated protein database searches with PSI-BLAST.
Cooper, P., NCBI/NLM, Bethesda, Maryland: NCBI resources for bioinformatics and computational biology; NCBI genome resources.
D'Eustachio, P., New York University Medical Center: The Reactome pathway database.
Hardison, R., Pennsylvania State University, University Park: Comparative genomics I: Tools for comparative genomics; Genome computation and gene regulation.
Pearson, W., University of Virginia, Charlottesville: Introduction and overview; Protein evolution and sequence similarity searching; Algorithms for biological sequence comparison; Hidden Markov models and protein profiles; Identifying consensus sites; Alignment algorithms: Large-scale alignment; Comparative genomics databases: Ensmart.
Smith, R., GlaxoSmithKline, King of Prussia, Pennsylvania: Approaches to multiple sequence alignment.
Yandell, M., HHMI/University of California, Berkeley: Genome annotation/sequence ontologies/gene ontologies; Large-scale computation on genomes.

Phage Display of Proteins and Peptides

November 9–22

INSTRUCTORS	**C. Barbas,** The Scripps Research Institute, La Jolla, California **D. Siegel,** University of Pennsylvania School of Medicine, Philadelphia **G. Silverman,** University of California, La Jolla
ASSISTANTS	**K. Masek,** Neurome Inc., La Jolla, California **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 was selected from peptide libraries and characterized.

The lecture series, presented by a number of invited speakers, emphasized 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. Seminar speakers in the 2005 course included Carl Goodyear (University of California, San Diego), Henry Lowman (Genentech, Inc.), Christopher Noren (New England Biolabs), Eric Ostertag (University of Pennsylvania School of Medicine), Sachdev Sidhu (Genentech, Inc.), George Smith (University of Missouri), Robyn Stanfield (The Scripps Research Institute), and K. Dane Wittrup (Massachussetts Institute of Technology).

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Aguilar, R., B.S., M.D. Anderson Cancer Center, Houston, Texas
Bar, M., B.S., Weizmann Institute of Science, Rehovot, Israel
Chao, D.-Y., Ph.D., Centers for Disease Control, Fort Collins, Colorado
Fiuza, U.-M., University of Cambridge, United Kingdom
Hong, Y., Ph.D., Tulane University Health Sciences Center, New Orleans, Louisiana
Huang, Y., Ph.D., Lawrence Livermore National Laboratory, California
Jayakumar, J., M.S., University of Capetown, South Africa
Lamkin, T., Ph.D., Air Force Research Laboratory, Brooks City-Base, Texas
Matoba, N., Ph.D., Arizona State University, Tempe, Arizona
Modin, C., Ph.D., University of Aarhus, Denamark
Muschler, J., Ph.D., California Pacific Medical Center, San Francisco
Norville, J., B.S., Massachusetts Institute of Technology, Cambridge
Razumenko, M., M.S., University of Trieste, Italy
Shallice, M., B.S., Invitrogen, Eugene, Oregon
Stolf, B., Ph.D., University of Sao Paulo, Brazil
Yeh, T.-M., Ph.D., National Cheng Kung University, Tainan, Taiwan

SEMINARS

Barbas, C., The Scripps Research Institute, La Jolla, California: Software and hardware for genomes: Polydactyl zinc finger proteins and the control of endogenous genes.
Goodyear, C., University of California, San Diego: Blymphocyte development and immunobiology.
Lowman, H., Genetech, Inc., San Francisco, California: SAR of peptides using phage.
Noren, C., New England Biolabs, Beverly, Massachusetts: Phage peptide libraries: The PhD for peptides.
Ostertag, E., University of Pennsylvania Medical School, Philadelphia: Use of phage display to clone human ADAMTS13 inhibitory antibodies from patients with thrombotic thrombocytopenic purpura.
Sidhu, S., Genetech, Inc., San Francisco, California: Antibody phage display and chemical diversity in antigen recognition.
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.
Smith, G., University of Missouri, Columbia: Phage display of peptides.
Stanfield, R., The Scripps Research Institute, La Jolla, California: Structural biology of the immune system.
Wittrup, K.D., Massachusetts Institute of Technology, Cambridge: Yeast display libraries.

The Genome Access Course

April 26–27, August 30–31, November 30–December 1

TRAINERS
J. Gergel, Cold Spring Harbor Laboratory
B. King, The Jackson Laboratory
P. Rabinowicz, The Institute for Genomic Research
D. Rasko, University of Texas Southwestern Medical Center
D. Schones, Cold Spring Harbor Laboratory

Initiated in 2002, this course is an intensive 2-day introduction to bioinformatics that was held three times in 2005 and trained 86 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. Applications to the course were open to all on a first-come-first-served basis, subject to basic eligibility requirements. The course was held at the Laboratory's Cancer Genome Research Center.

April 26–27

Barria, A., Ph.D., Cold Spring Harbor Laboratory
Bogusz, A. Ph.D., University of Chicago, Illinois
Bohling, K., B.S., Iowa State University
Bouck, A., Ph.D., Duke University, Durham, North Carolina
Catron, S., Ph.D., Pioneer Hi-Bred International, Johnston, Iowa
Chen, H., Ph.D., University of Maryland School of Medicine, Baltimore
Cuenco, K.T., Ph.D., University of Maryland School of Medicine, Baltimore
Deigendesch, N., B.S., Massachusetts Institute of Technology, Cambridge
Friedlaender, F., Ph.D., Temple University, Philadelphia, Pennsylvania
Friedlaender, J., Ph.D., Temple University, Philadelphia, Pennsylvania
Fuller, B., Ph.D., University of Virginia, Charlottesville
Gao, S., Ph.D., State University of New York, Stony Brook

Gould, H., Ph.D., King's College, London, United Kingdom
Kramnik, I. Ph.D., Harvard School of Public Health, Boston, Massachusetts
Lee, T., Ph.D., University of Toronto, Canada
Li, R., Ph.D., Sam Houston State University, Huntsville, Texas
Muratani, M., B.S., Cold Spring Harbor Laboratory
Peled, J., B.S., Albert Einstein College of Medicine, Bronx, New York
Plourde, M., B.S., Université Laval, Québec, Canada
Rao, C.P., Ph.D., St. Luke's Memorial Hospital, Utica, New York
Ronai, D., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Sprague, G., Ph.D., University of Oregon, Eugene
Su, X., Ph.D., Cedars-Sinai Medical Center, Los Angeles, California
Tange, T., Ph.D., Howard Hughes Medical Institute, Waltham, Massachusetts
Teplin, S., Ph.D., Cold Spring Harbor Laboratory
Teshima, K., Ph.D., Brown University, Providence, Rhode Island
Tewari, D., Ph.D., Pennsylvania Department Agriculture, Harrisburg, Pennsylvania
Wang, L., Ph.D., Vanderbilt University, Nashville, Tennessee
Yasenchak, J., Regeneron Pharmaceuticals, Tarrytown, New York
Zhai, B., Ph.D., CBRC, Massachusetts General Hospital, Charlestown

August 30–31

Barretina Ginesta, J., Ph.D., Dana-Farber Cancer Institute, Boston Massachusetts
Bartfai, R., Ph.D., Temasek Life Sciences Laboratory, Singapore
Burns, F., Ph.D., Dupont/Qualicon, Wilmington, Delaware
Caufield, P., Ph.D., New York University
Cohen, E., Ph.D., The Salk Institute for Biological Studies, La Jolla, California
Costa-Guda, J., B.S., University of Connecticut Health Center, Farmington
Cui, H., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Das, B., Ph.D., Carnegie Institution of Washington, Baltimore, Maryland
Davis, R., Ph.D., SUNY Upstate Medical University, Syracuse, New York
DeMayo, F., Ph.D., Baylor College of Medicine, Houston, Texas
Doucet, J., Ph.D., Nicholls State University, Thibodaux, Louisiana
Echlin, T., B.S., National Institutes of Health, Bethesda, Maryland
Guan, X., Ph.D., Syngenta Biotechnology Inc., Durham, North Carolina
Horton, M., B.S., University of Chicago, Illinois
Isaac, D., Ph.D., Princeton University, New Jersey
Keller, A., B.S., The Rockefeller University, New York
King, M., Ph.D., University of Rochester, New York
Lauter, K., B.S., University of Connecticut, Farmington
Lim, K.-C., Ph.D., University of Michigan Medical School, Ann Arbor
Moran, U., Ph.D., Icon Laboratories, Farmingdale, New York
Morrow, E., Ph.D., Harvard Medical School, Boston, Massachusetts
Nasipak, B., B.S., Columbia University, New York
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November 30–December 1

Bain, P. Ph.D., Harvard Medical School, Boston, Massachusetts
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Martinelli, D., B.S., Carnegie Institution of Washington, Baltimore, Maryland
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Molinaro, A., Ph.D., Yale University, New Haven, Connecticut
Monroe, J., Ph.D., James Madison University, Harrisonburg, Virginia
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O'Donnell, J., B.S., Pathfinder Regional Vocational Technical School, Palmer, Massachusetts
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Temple, L., Ph.D., James Madison University, Harrisonburg, Virginia
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Wilt, S., Ph.D., Bellarmine University, Louisville, Kentucky

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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.

	Title	Host
January		
Dr. Michael Grunstein, University of California, Los Angeles	Functions of histone acetylation sites in yeast.	Terri Grodzicker
Dr. David Livingston, Dana Farber Cancer Institute, Harvard University	Functional analysis of the *BRCA1* gene and its role in tumor suppression.	Terri Grodzicker
Dr. Marc Tessier-Lavigne, HHMI, Stanford University	Wiring the brain: The logic and molecular biology of axon guidance.	Grisha Enikolopov
February		
Dr. Joel Richter, University of Massachusetts Medical School	Translational control of developmental and neuronal plasticity.	Holly Cline
Dr. Josh Sanes, Harvard University	Analyzing synapse formation with mutant and fluorescent mice.	Alla Karpova (Svoboda Lab)
Dr. David Sinclair, Harvard Medical School	Unraveling the regulators of aging: From plants to yeast to mammals.	William Tansey
Dr. Yuh-Nung Jan, HHMI, University of California, San Francisco	Control of dendrite morphology and remodeling during development.	Linda Van Aelst
March		
Dr. Walter Mangel, Brookhaven National Laboratory	Activation of the adenovirus proteinase by a long, branched signal transduction pathway within the protein: Targets for antiviral drugs.	Rui-Ming Xu
Dr. Steve McKnight, Southwestern Medical Center, The University of Texas	Schizophrenia, stem cells, and sprouty signaling.	Bruce Stillman
Dr. Ruth Lehmann, Skirball Institute, New York University	Germ cells are forever: Germ-line stem cell migration and development.	Cordula Schulz
Dr. Jeffrey Pessin, Stony Brook University	Intracellular trafficking of the insulin responsive glucose transporter.	Holly Cline
September		
Dr. Charalambos Kyriacou, University of Leicester, U.K.	Molecular biology of the circadian clock.	Tim Tully
October		
Dr. Louis F. Reichardt, University of California, San Francisco	Roles of cadherins and catenins and their signaling pathways in synapse development.	Josh Huang
November		
Dr. Stephen L. Mayo, HHMI, California Institute of Technology	Modulation and de novo design of protein–protein interactions.	Leemor Joshua-Tor
Dr. Lily Y. Jan, HHMI, University of California, San Francisco	Potassium channels.	David Mu
Dr. George Yancopoulos, Executive Vice President and Chief Scientific Officer, Regeneron Pharmaceuticals, Inc.	VEGF trap in cancer trials today, and VelociGene coupled with VelocImmune for drug targets of tomorrow.	Terri Grodzicker

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.

	Title
January	
Jonathan Sebat (Wigler Lab)	Large-scale copy-number variation: A ubiquitous characteristic of the human genome and a cause of common genetic diseases.
Bruce May (Martienssen Lab)	Polar express: Centromeres and heterochromatin in *Arabidopsis*.
Jack Chen (Stein Lab)	Analysis of chemosensory genes in *Caenorhabditis elegans*.
Cordula Schulz	Cell intrinsic and extrinsic pathways regulate stem cell function in the *Drosophila* male gonad.
February	
Senthil Muthuswamy	Genesis of carcinoma: Does shape matter?
Alea Mills	A new look at the role of the *p53* homolog *p63* in development, cancer, and aging.
March	
Sandra Kuhlman (Huang Lab)	GABAergic circuits and the regulation of critical period plasticity.
Guido Wendel (Lowe Lab)	Tumor suppressors and chemotherapy response.
Christian Speck (Stillman Lab)	License to replicate DNA–00(7) proteins in action.
April	
Andrew Smith (Zhang Lab)	Computational pattern discovery in regulatory sequences.
October	
Rob Martienssen	Making sense of junk RNA.
Jim Hicks (Wigler Lab)	Breast cancer genomics: Studies of 250 clinical cases using ROMA microarray analysis.
Kathy Tworkowski (Tansey Lab)	A novel oncogenic mechanism to control Myc proteolysis.
November	
Anthony Holtmaat (Svoboda Lab)	Imaging experience-dependent structural plasticity in the mouse neocortex.
Namiko Satoh-Nagasawa (Jackson Lab)	Regulation of maize architecture: A sweet solution.
Laurence Denis (Spector Lab)	Establishing the histone H3 K9 methylation pattern during S phase.
December	
Graziella di Cristo (Huang Lab)	Construction of GABAergic circuits in visual cortex: Role of genes and experience.
Vivek Mittal	Vascular progenitors control angiogenesis-mediated tumor growth.
Lars Zender (Lowe Lab)	An integrative approach to cancer genetics and cancer biology: Cross-species comparison of liver cancer in mice and men.

BANBURY CENTER

BANBURY CENTER
EXECUTIVE DIRECTOR'S REPORT

The year 2005 was remarkable for the Banbury Center, with record numbers of events and participants. The number of scientific meetings rose 33% from 2004, from 18 to 24, and the Center was used for 11 other events, for a total of 35. The increase in participants paralleled the increase in meetings, with 806 participants compared to 568 in 2004. The proportion of participants from the United States remained the same at 80%, drawn from 35 states, with New York, California, Massachusetts, and Maryland leading the way. Foreign participants came from 30 countries, and we were particularly pleased to welcome participants from eight African countries who came for the Albert B. Sabin vaccine meeting.

There were three very significant staffing changes in 2005. Katya Davey retired after 26 years as hostess at Robertson House. Katya was one of the first staff members at Banbury and is known to tens of thousands of scientists throughout the world for her kindness and helpfulness. She has been an icon of the Banbury Center and her character and personality will be greatly missed. We were fortunate in finding Barbara Polakowski who now has the formidable task of looking after the scientists staying on the Banbury estate. The third of the changes was the appointment of Sydney Gary as Assistant Director. She is a neuroscientist who worked with Susan Hockfield at Yale. Sydney has come to develop the neuroscience and mental health meetings and courses programs at Banbury and on the main campus.

Katya Davey

One meeting at Banbury in 2005 broke new ground for Banbury Center meetings, and for Cold Spring Harbor Laboratory in general. Mila Pollack, Director of Libraries and Archives here at the Laboratory, and Darwin H. Stapleton, Director of the Rockefeller Archives, organized a meeting on *History of Science: Archives and Oral History.* This was the first occasion on which a history of science meeting has been held here and it was a great success. Participants reviewed contentious issues such as the preservation of letters and communications in the age of e-mail, and whether archives should be digitized and made available via the Internet. They also described how they deal with these issues at their own institutions. The discussion meeting was particularly useful in helping to promote the Laboratory's initiative in developing a history of molecular biology and molecular genetics program, building on the collection donated by Jim Watson.

Five meetings were held relating to cancer, four dealing with specific types of cancer and the fifth covering a new and potentially very important topic: cancer stem cells. Despite the fact that B-cell chronic lymphocytic leukemia (B-CLL) is the most common leukemia in the western hemisphere, the cause of the disease remains enigmatic and the treatments inadequate. However, recent advances using a variety of techniques and approaches led to a meeting, *Chronic Lymphocytic Leukemia*, organized by Nicholas Chiorazzi and Kanti R. Rai (Institute for Medical Research, North Shore–LIJ Health System) and Michael Wigler (Cold Spring Harbor Laboratory). Participants included investigators using immunological, molecular biological, and genetic approaches, as well as physicians with expertise in the study and treatments of B-CLL patients. The meeting structure was ambitious, with multiple short presentations providing an opportunity for participants to contribute to more than one session.

Banbury Center conference room, summer

The Biology of Neuroendocrine Tumors, organized by Arnold J. Levine (Institute for Advanced Studies) and Evan Vosburgh (Verto Institute), focused on several topics: Genetic studies using whole-genome allelotyping, SNPS in the *p53* pathway, and LINE1 retrotransposon studies of human cell lines and tumor samples. These

are beginning to identify specific genetic regions of interest and to characterize the genomic instability of this class of tumors. The roles of the tumor suppressors, menin and parafibromin, were discussed. Menin is involved in signaling pathways, proliferation, and cell death, whereas parafibromin is associated with RNA polymerase and plays a part in histone modifications. Translational research on EGFR and VEGF/VEGFr was complimented by presentations of early clinical studies of anti-EGFR and anti-VEGF therapies.

Kevin M. Shannon (University of California, San Francisco) and Kim Hunter-Schaedle (Children's Tumor Foundation) were the organizers of *Barriers and Solutions in the Use of Mouse Models to Develop Therapeutic Strategies for NF1- and NF2-associated Tumors*. Mice are used extensively for the development and assessment of cancer therapies, but there are questions about how best to model human tumors in mice. Participants examined the advantages, limitations, and potential new directions of using mouse models of neurofibromatosis 1 and neurofibromatosis 2 in developing therapeutics for neurofibromatosis.

Dorothea Becker (University of Pittsburgh) and Martin McMahon (University of California, San Francisco) organized *A Critical Review of Melanoma: Genomic Approaches with Therapeutic Promise*. The meeting brought together investigators who are using array profiling, SAGE technology, proteomics, and optical imaging, as well as basic scientists and physicians who focus on the identification and characterization of genes that govern important functions in early and advanced-stage melanoma and precursor lesions. The goal of the meeting was to outline new strategies for gaining further insights into the molecular pathways of melanoma in light of a critical review of current research and targeted therapies for melanoma.

An important new area of cancer research concerns whether stem cells present in adult tissues might be the sources of cancers. This is not a new idea and was a popular theory of cancer at the end of the 19th century when it was proposed that embryonic cells, "rests," persisted into the adult and that these cells could be reactivated and grow in the adult. Now, we have the molecular tools and the intellectual background to reinvestigate this idea, as was evident in the *Cancer Stem Cells* meeting, organized by Max Wicha (University of Michigan) and Jeffrey M. Rosen (Baylor College of Medicine). Discussions ranged from signaling pathways investigated in embryos, through what is known of stem cells in leukemias and other cancers, to how this knowledge could change current approaches to cancer treatments. Current cancer therapies, which have been developed on the basis of their ability to cause tumor regression, might selectively target these differentiated cells and spare the cancer stem cell component. The latter may contribute to a tumor recurrence.

Banbury Center held a meeting on scientific fraud in 1989 and a meeting on nuclear transfer (cloning) in 2000. I had not thought that the two topics would come together, but circumstances following the 2005 meeting, *The Biology and Practice of Mammalian Cloning: A Reassessment*, dictated otherwise. Organized by Peter Mombaerts (The Rockefeller University) and Ian Wilmut (University of Edinburgh), it was a follow-up to the meeting on cloning of mammals held in 2000, just 4 years after the cloning of Dolly. Topics covered included nuclear transfer experiments in mice, rats, rabbits, and cattle, as well as human beings. We were very pleased that the leading exponent of nuclear transfer in human beings, Woo-Suk Hwang from South Korea, was participating. Now it appears that all of the work done by Hwang's laboratory on human embryonic stem cells was fabricated. There is no doubt that the basic premise and promise of human stem cell therapy were not diminished by this scandal but it is a setback to the field.

Banbury continues to hold meetings on human genetic disorders because much needs to be done to capitalize on the disease gene discoveries that were so successful in the 1990s. Therapies are still elusive for so many of these disorders. *Translational Approaches to Fragile-X Syndrome: Turning Basic Research Findings into Therapeutic Targets* (organized by Elizabeth Berry-Kravis, Rush Children's Hospital; William T. Greenough, University of Illinois; and Katie Clapp, FRAXA Research Foundation) tackled this issue head-on. The participants focused on strategies for translation of basic science knowledge about phenotypes and therapeutic targets in Fragile-X syndrome and its animal models to clinical treatment trials in patients. They discussed how to determine treatment targets and methods of assessing the efficacy of treatments in animal models.

The meeting, *Spinal Muscular Atrophy: Neuronal Rescue and Repair from Laboratory to Clinic,* examined the potential of therapies and how they might be made available. Held at Banbury, March 13–16, the organizers were Loren Eng (Spinal Muscular Atrophy Foundation, New York), Thomas M. Jessell (Columbia University), Alex E. MacKenzie (Children's Hospital of Eastern Ontario), Kay E. Davies (University of Oxford), and Cynthia Joyce (SMA Foundation). The discussions ranged widely from the biochemistry and cell biology of SMA and what these can tell us of potential drug targets, to SMA genetics and how this knowledge can be used in the development of animal models, and, finally, to a discussion of strategies.

Researchers and clinicians working on amyotrophic lateral sclerosis (ALS) are investigating the therapeutic potential of stem cells. Lucie Bruijn (The ALS Association), Stephen M. Strittmatter (Yale University), and Clive N. Svendsen (University of Wisconsin) organized the meeting *Stem Cells and Axonal Regeneration: Strategies for the Treatment of ALS.* In vitro studies have been carried out examining stem cells that may differentiate and replace dying neurons and/or sick astrocytes in SMA. Another approach might be to stimulate the proliferation of endogenous stem cells to replace dying cells. Participants also discussed how any new cells would establish appropriate connections and reviewed current knowledge of axonal guidance cues.

The genetics of epilepsy are not as advanced as those for Fragile-X or SMA, but the mechanisms of drugs that have empirically been shown to be effective in epilepsy may provide leads. Norman Delanty (Royal College of Surgeons in Ireland), David B. Goldstein (Duke Institute for Genome Sciences and Policy), Ley Sander (University College, London), and Sanjay M. Sisodiya (University College London) organized *Epilepsy Genetics and Pharmacogenetics*. Some new developments, including the availability of HapMap data and a growing understanding of the action of many anti-epileptic drugs, made this the right moment to bring together those involved in different aspects of epilepsy genetics to identify research priorities and strategies, and to foster collaborations among groups with different clinical resources.

Parkinson's disease (PD) is one of the most common movement disorders, afflicting individuals from all walks of life. It is becoming the subject of increasing research because of the severity of the disorder and increasing public awareness. *Parkinson's Disease: Basic Mechanisms and Therapies* (organized by Rodolfo Llinas, New York University Medical Center, and Ali Rezai, The Cleveland Clinic Foundation) critically reviewed the latest findings on the pathophysiology of PD, therapies based on drug treatment and, most especially, the degree to which surgical interventions ameliorate the movement disturbances.

Mitochondria are the powerhouses of cells, and, not surprisingly, illness results when they fail or work inefficiently. It is becoming clear that mitochondria may be involved in a much broader range of disorders than had been suspected, and *Mitochondria in Neurological Disease and Aging* critically reviewed the evidence for mitochondrial involvement in these processes. Organized by M. Flint Beal (Cornell University) and Douglas C. Wallace (University of California, Irvine), the topics ranged from research on the biochemistry and physiology of mitochondria, through animal models of mitochondrial disease, to specific diseases, including Friedreich ataxia, ALS, Huntington's disease, Parkinson's disease, and Alzheimer's disease.

Banbury Center conference room, winter

One of the most fascinating stories in modern biology concerns prions, abnormal proteins with no associated DNA or RNA that can be transmitted among animals. The diseases they cause were known as scrapie in sheep and wasting disease in elk, but they came to public attention when, in the early 1980s, cases of a formerly very rare human disorder—Creutzfeld-Jakob disease (CJD)—appeared in the United Kingdom. Investigation showed that these were a variant form of CJD (vCJD) and were caused by prions that had been transmitted to human beings who ate contaminated beef. Much still remains mysterious about prions, and we held a meeting *Prion Biology: Puzzles and Paradoxes* organized by John Collinge (University College, London) and Charles Weissmann (The Scripps Institute).

There was extended discussion about whether synthetic prions, which are devoid of nucleic acid, have been made, and needless to say, much still remains to be resolved. The meeting did set a new record for a Banbury Center meeting—three of the participants, 12%, were Nobel laureates!

The Banbury conference on *The GABAergic System* was organized by Josh J. Huang (Cold Spring Harbor Laboratory) and György Buzsáki (Rutgers University) and took place October 30–November 2. GABA is one of the major neurotransmitters and the only mediator of inhibition in the brain. GABA dysfunction has been implicated in a range of neurological, neurodevelopmental, and psychiatric disorders. Progress in understanding the genetic design, construction, and mode of operation of the GABAergic system will significantly advance our knowledge of brain development and function. The Banbury meeting brought together scientists using molecular and genomic approaches, and developmental neurobiologists, physiologists, system neuroscientists, and clinicians to foster future work in this critical domain of research.

The most fascinating area of neuroscience research concerns the interface between biology and higher-level processes. *Neurobiology of Decision-making,* organized by Carlos Brody (Cold Spring Harbor Laboratory), Michael N. Shadlen (University of Washington), and Xiao-Jing Wang (Brandeis University), considered such questions as: What are the key computations involved in making a decision? How are they implemented by neurons? Can we quantitatively formulate the probabilistic nature of decision-making? What is the predominant source of randomness intrinsic to the brain? Can the importance of noise in decision-making behavior be tested experimentally? The meeting included investigators who employ high-level abstract mathematical psychology descriptions, neural network modeling, and experimental approaches.

The theme of *The Intracellular Molecular Environment* (David Spector, Cold Spring Harbor Laboratory, and Jason Swedlow, University of Dundee) can be summarized in the rather naive question: What would it be like to be a molecule inside the cell? And from this question, others arise: How crowded would it be? How would I get to where I needed to be? If I am part of a complex, how do I find the members of the complex? How do data from in vitro experiments relate to similar processes in the cell? The meeting brought together scientists who use theoretical and experimental analyses employing a variety of techniques, including biophysical, biochemical, mathematical, imaging, and cell biological experimentation.

Plants are remarkable in that they are frequently polyploid, i.e., they have two or more copies of their genome. Such polyploidy is important for plant functions and evolution. *Polyploidy, Heterosis, and Genomic Balance* was organized by Jim A. Birchler (University of Missouri, Columbia) and Luca Comai (University of Washington). The participants discussed gene expression in aneuploidy, polyploidy, or hybrid states (heterosis and species hybrids); the molecular basis of quantitative traits; gene regulatory circuits; the relationship of epigenetic phenomena to gene regulatory mechanisms; and the evolution of gene regulation.

There were four meetings related to infectious diseases. A rather unusual topic of a Banbury Center meeting was *Computational Approaches for Biomarker Discovery* (Suzanne Vernon and William Reeves, Centers for Disease Control and Prevention). Hiding behind this rather unremarkable title was a fascinating meeting on a Centers for Disease Control project examining chronic fatigue syndrome (CFS). This is not an easy subject to study—diagnosis is difficult, laboratory diagnostic tests are not available, and multiple factors likely contribute to its pathogenesis. CDC has examined a large cohort of individuals from Wichita Falls, subjecting them to a battery of physical, psychiatric, and laboratory tests, including gene expression analysis using microarrays. The expectation is that analysis of this mass of data will reveal patterns common to individuals with CFS. However, this is not a simple task—it requires the integration of many different kinds of data. Participants included mathematicians, engineers, computer scientists, as well as biologists, trying to develop the most informative ways of handling the data.

Bioterrorism continues to be an important topic, and Banbury had two meetings on viral and microbial forensics. The title of the first meeting, *Pathogenesis and Early Events in Viral Infection* (organized by Roger Breeze and Floyd Horn, Institute for Comparative Genomics; William Laegreid, USDA; and Daniel L. Rock, University of Illinois), would not have been out of place for the Laboratory in the 1970s,

when tumor viruses such as SV40 and adenovirus were the subjects of intense research here. However, the viruses that were the focus of attention for this meeting would not have been studied at Cold Spring Harbor. They included West Nile virus, rinderpest, and dengue, ebola, and Marburg hemorrhagic fever viruses. The second meeting dealt with an ostensibly much more prosaic topic: *Microbial Forensics 2005: Sample Management* (organized by Steven Schutzer, UMDNJ; Bruce Budowle, Federal Bureau of Investigation; and James P. Burans, U.S. Department of Homeland Security). However, procedures that are commonplace in ordinary investigations (e.g., maintaining chain of custody) may be unfamiliar or difficult to achieve with biological samples. Or suppose that a test or extraction procedure for one chemical precludes other tests. How should priorities be determined?

The Albert B. Sabin Foundation Colloquium on *Introduction and Sustainable Use of Vaccines in Developing Countries* was organized by Kevin Reilly (Sabin Vaccine Institute) and Francisco F. Songane (Ministerio da Saude, Mozambique). Participants reviewed the most critical issues facing financiers, government, industry, and the developing countries that are in need of funds for vaccine purchase and delivery; defined the performance criteria that will help benefactors and beneficiaries measure the progress of the developing countries; and made realistic recommendations that best enhance the prospects for sustained use of needed vaccines in developing countries. A highlight of the meeting was a talk by Sir George Alleyne of the Pan American Health Organization, Washington, D.C.

Two groups took the opportunity of Banbury meetings to hold associated policy meetings. The National Cancer Institute–International Workshop on CLL Group and the NSF Polyploidy Group held premeetings.

In addition to the meetings, Banbury hosted several courses. There were the regular summer courses and a 2-week workshop on schizophrenia. The Watson School of Biological Sciences held two Topics in Biology courses for its students. Banbury also hosted two courses for outside groups. The Boehringer Ingelheim Foundation held a course on the conduct of science for its North American fellows, and David Micklos and I, together with Ken Culver, provided a genetics course for part of the oncology group of Novartis.

Sydney Gary and Barbara Polakowski

The Banbury Center continues to make a unique contribution to the world of biology, fulfilling the goals and aspirations set by Charles Robertson and Jim Watson almost 30 years ago. It does so through the efforts of many people at Cold Spring Harbor: Bea Toliver, Ellie Sidorenko, Sydney Gary, Barbara Polakowski, Chris McEvoy, and Joe Ellis at Banbury, and the staff of audiovisual, the meetings office, and the housekeeping department, and Blackford.

Jan Witkowski
Executive Director

Parkinson's Disease: Basic Mechanisms and Therapies

January 16–18

FUNDED BY **The Thomas Hartman Foundation For Parkinson's Research**

ARRANGED BY **R. Llinas**, New York University Medical Center
A. Rezai, The Cleveland Clinic Foundation

Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory
R. Llinas, New York University Medical Center
A. Rezai, The Cleveland Clinic Foundation

SESSION 1: Morphology and Anatomical Basis of Parkinson's Disease
Chairperson: A. Beric, Hospital for Joint Diseases, New York

E.G. Jones, University of California, Davis: Cortical-thalamic pathways.

A. Graybiel, Massachusetts Institute of Technology, Cambridge: Cortical-basal ganglia-thalamic pathways.

SESSION 2: Physiology and Pathophysiology: Thalamus, Basal Ganglia, and Cortex
Chairperson: A.-L. Benabid, l'Universite Joseph Fourier, Grenoble, France

R. Llinas, New York University Medical Center: Thalamo-cortical rhythm: Function and pathology.

A.M. Lozano, Toronto Western Hospital Research Institute, Canada: Neurophysiological attributes of Parkinson's disease: Insights from intraoperative single neuronal recordings.

F. Lenz, The Johns Hopkins Hospital, Baltimore, Maryland: Feedback control in Parkinsonian symptoms of tremor and dystonia: Abnormal gain and phase of thalamic transfer functions.

R. Llinas, A. Grabil, B. Kopell

SESSION 3: Diagnostics and Imaging of Parkinson's Disease
Chairperson: R. Llinas, New York University Medical Center

J. Volkmann, Christian-Albrechts-University, Kiel, Germany: Clinical neurophysiology of the interaction between basal ganglia, brain stem, and spinal pathways in Parkinson's disease.
D. Eidelberg, North Shore University Hospital, Manhasset, New York: Imaging and pathophysiology.
A.Y. Mogilner, North Shore University Hospital, Manhasset, New York: Anatomic imaging and surgical targeting.
B. Kopell, Medical College of Wisconsin, Milwaukee: The postoperative role in imaging in DBS and neurostimulation surgery.

SESSION 4: Surgery for Parkinson's Disease
Chairperson: A. Rezai, The Cleveland Clinic Foundation, Ohio

D. Jeanmonod, University Hospital Zurich, Switzerland: Targets and role of lesioning.
A.-L. Benabid, l'Universite Joseph Fourier, Grenoble, France: Targets and electrical stimulation.
A.M. Lozano, Toronto Western Hospital Research Institute, Canada: Intraparenchymal brain delivery of therapeutic compounds for Parkinson's disease.
M.G. Kaplitt, Weill Medical College of Cornell University, New York/N. Boulis, The Cleveland Clinic Foundation, Ohio: Targets and gene therapy/neurotransplantation.

SESSION 5: Emerging Surgical Approaches for Neurological and Psychiatric Disorders and Next Steps
Chairpersons: R. Llinas, New York University Medical Center; **A. Rezai,** The Cleveland Clinic Foundation, Ohio

B.D. Greenberg, Butler Hospital, Providence, Rhode Island: Surgery for psychiatric disorders.
A. Rezai, The Cleveland Clinic Foundation, Ohio: Surgical intervention: Emerging technology and applications—Chronic pain, cluster headaches, epilepsy, stroke, Tourette's aggression, obesity.

Summary/Future Steps

Chronic Lymphocytic Leukemia

February 6–9

FUNDED BY **The Karches Foundation**

ARRANGED BY **N. Chiorazzi,** The Institute for Medical Research, North Shore–LIJ Health System
K.R. Rai, The Institute for Medical Research, North Shore–LIJ Health System
M. Wigler, Cold Spring Harbor Laboratory

Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory
B. Stillman, Cold Spring Harbor Laboratory
N. Chiorazzi, The Institute for Medical Research, North Shore–LIJ Health System, Manhasset, New York

SESSION 1
Chairperson: N. Chiorazzi, The Institute for Medical Research, North Shore–LIJ Health System, Manhasset, New York

K.R. Rai, The Institute for Medical Research, North Shore–LIJ Health System, New Hyde Park, New York: B-CLL and the major unanswered questions.
P. Hillmen, Pinderfields Hospital, Wakefield, United Kingdom: "Preleukemic" cells that circulate in normal individuals.
F. Caligaris-Cappio, Universita Vita-Salute San Raffaele, Milano, Italy: Monoclonal $CD5^+$ and $CD5^-$ B-cell expansions in the peripheral blood of the elderly.
M.D. Cooper, HHMI, University of Alabama, Birmingham: B-lymphocyte development and subsets in man.
J. Monroe, University of Pennsylvania School of Medicine, Philadelphia: B-cell development.
R. Davis, University of Alabama, Birmingham: Biological potential of Fc receptor homologs on B lineage cells.
F. Caligaris-Cappio, Universita Vita-Salute San Raffaele, Milano, Italy: CLL cells think B and speak T.
M. Ferrarini, Instituto Nazionale per la Ricerca sul Cancro, Genova, Italy: The B-CLL cell: Identification and characteristics.
U. Klein, Columbia University, New York: Is the normal cell equivalent of CLL a memory cell?
F. Caligaris-Cappio, Universita Vita-Salute San Raffaele, Milano, Italy: Expression of ZAP-70 in normal human mature B cells.

SESSION 2
Chairperson: E. Montserrat, University of Barcelona, Spain

J. Monroe, University of Pennsylvania School of Medicine, Philadelphia: Positive and negative selection of the B-cell repertoire.
N. Chiorazzi, The Institute for Medical Research, North Shore–LIJ Health System, Manhasset, New York: Structure of the BCR in B-CLL cells.
F. Caligaris-Cappio, Universita Vita-Salute San Raffaele, Milano, Italy: Geographical patterns and pathogenetic implications of IGHV3-21 gene usage.

J. Monroe, M. Keating, T. Kipps

T.J. Kipps, University of California, San Diego: Function of the BCR in B-CLL cells.
E. Montserrat, University of Barcelona, Spain: ZAP-70 expression and prognosis in B-CLL.
M. Ferrarini, Instituto Nazionale per la Ricerca sul Cancro, Genova, Italy: Differential triggering through the BCR in B-CLL.
T. Honjo, Kyoto University, Japan: BCR signaling and AID function.

SESSION 3
Chairperson: T.J. Kipps, University of California, San Diego

S. Lowe, Cold Spring Harbor Laboratory: Apoptosis.
T.J. Kipps, University of California, San Diego: Apoptosis in B-CLL.
F. Caligaris-Cappio, Universita Vita-Salute San Raffaele, Milano, Italy: Role of T cell and cytokines in apoptosis regulation in B-CLL.
M. Ferrarini, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy: Apoptosis in B-CLL.
N. Kay, Mayo Clinic, Rochester, Minnesota: Apoptosis and green tea extract in B-CLL.

SESSION 4
Chairperson: N. Kay, Mayo Clinic, Rochester, Minnesota

N. Kay, Mayo Clinic, Rochester, Minnesota: Microenviromental considerations in B-CLL.
H. Schreiber, University of Chicago, Illinois: Role of the microenvironment in supporting lymphoid cell development and growth.
M. Lipp, Max Delbrück Center for Molecular Medicine, Berlin, Germany: Role of chemokines and cytokines in supporting lymphoid cell development and growth.
F. Caligaris-Cappio, Universita Vita-Salute San Raffaele, Milano, Italy: Role of the microenvironment in supporting B-CLL cell development and growth.
T.J. Kipps, University of California, San Diego: Role of nurse cells in nurturing B-CLL cells.
M. Lipp, Max Delbrück Center for Molecular Medicine, Berlin, Germany: Ectopic lymphoid follicle formation.

SESSION 5
Chairperson: M. Wigler, Cold Spring Harbor Laboratory

U. Klein, Columbia University, New York: Gene expression profiling in B-CLL and other B-cell lymphoproliferative disorders.
J.G. Gribben, Barts and Royal London School of Medicine, United Kingdom: Gene expression profiling of T cells in B-CLL.
M. Keating, M.D. Anderson Cancer Center, Houston, Texas: Difference in gene expression between B-CLL and normal B cells.
C.M. Croce, Ohio State University Medical Center, Columbus: Role of micro-inhibitory RNAs in B-CLL.
L. Pasqualucci, Columbia University, New York: Role of Bel-6 in non-Hodgkin's lymphoma.

SESSION 6
Chairperson: P. Hillmen, Pinderfields Hospital, Wakefield, United Kingdom

M. Wigler, Cold Spring Harbor Laboratory: Genome-wide genomic screening using ROMA.
P. Lichter, German Cancer Research Center, Heidelberg, Germany: Genome-wide screening in B-CLL.
C.M. Croce, Ohio State University Medical Center, Columbus: Tel-1 in B-CLL.
M. Wabl, University of California, San Francisco: Murine leukemia virus system to generate genomic deletions.
H. Schreiber, University of Chicago, Illinois: Inflammation as a tumor promoter in cancer induction.

SESSION 7
Chairperson: C.M. Croce, Ohio State University Medical Center, Columbus

T. Honjo, Kyoto University, Japan: Activitation-induced cytidine deaminease: Structure and function.
M. Nussenzweig, The Rockefeller University, New York: Activation-induced cytidine deaminase: Structure and function.
M.D. Cooper, HHMI, University of Alabama, Birmingham: Somatic diversification of variable lymphocyte receptors in lower species.
N. Chiorazzi, Institute for Medical Research North Shore–LIJ Heath System, Manhasset, New York: AID expression in B-CLL.
M. Keating, M.D. Anderson Cancer Center, Houston, Texas: Expression of AID and its splice variants in B-CLL.
M. Wabl, University of California, San Francisco: Genome-wide somatic hypermutation.
L. Pasqualucci, Columbia University, New York: Role of somatic hypermutation in the pathogenesis of B-cell neoplasms.

SESSION 8

Chairperson: S.L. Allen, The Institute for Medical Research, North Shore–LIJ Health System, Manhasset, New York

M. Hallek, Universitaet zu Koeln, Germany: History and state of the art of pharmacologic therapy.

M. Keating, M.D. Anderson Cancer Center, Houston, Texas: Monoclonal antibody therapy in B-CLL.

P. Hillmen, Pinderfields Hospital, Wakefield, United Kingdom: Monoclonal antibody therapy in B-CLL.

R. Berenson, Xcyte Therapies, Inc., Seattle, Washington: Adoptive T-cell immunotherapy.

R. Damle, Institute for Medical Research North Shore–LIJ Health System, Manhasset, New York: Telomere length and telomerase expression in B-CLL cells.

C.B. Harley, Geron Corporation, Menlo Park, California: Telomerase inhibition, cancer, and GRN163L.

M. Lipp, Max Delbrück Center for Molecular Medicine, Berlin, Germany: Chemokine receptors as potential therapeutic targets.

SESSION 9

Chairperson: K.R. Rai, The Institute for Medical Research, North Shore–LIJ Health System, New Hyde Park, New York

D. Valmori, Columbia University, New York: Vaccination in B-CLL.

T.J. Kipps, University of California, San Diego: Gene therapy in B-CLL.

M. Hallek, Universitaet zu Koln, Germany: Use of adeno-associated viral vectors for gene therapy.

J.G. Gribben, Barts and Royal London School of Medical, United Kingdom: Stem cell transplantation in B-CLL.

E. Montserrat, University of Barcelona, Spain: Allo- and auto-transplantation in B-CLL.

T. Honjo, Kyoto University, Japan: Role of PD-1 in tumor immunity.

Translational Approaches to Fragile-X Syndrome: Turning Basic Research Findings into Therapeutic Targets

February 27–March 2

FUNDED BY **NIH–National Institute of Mental Health (through a grant to the University of Illinois)**

ARRANGED BY **E. Berry-Kravis**, Rush Children's Hospital
W.T. Greenough, University of Illinois
K. Clapp, FRAXA Research Foundation

Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Phenotype of Fragile X
Chairperson: R.J. Hagerman, University of California, Davis Health System, Sacramento

K. Clapp, FRAXA Research Foundation, Newburyport, Massachusetts: A parent's perspective on Fragile X.
M.R. Tranfaglia, FRAXA Research Foundation, Newburyport, Massachusetts: Psychiatric symptoms of Fragile X.
W.T. Greenough, University of Illinois, Urbana: Neuroanatomical phenotype of FXS and role of the absence of FMRP.
R.E. Paylor, Baylor College of Medicine, Houston, Texas: Behavioral phenotypes in mouse model of Fragile X.
B.A. Oostra, Erasmus Universiteit Rotterdam, The Netherlands: Eye-blinking experiments in mice and humans.
K. Broadie, Vanderbilt University and Medical School, Nashville, Tennessee: A *Drosophila* model of Fragile-X syndrome.

J. Weiler, J. Lauterborn

SESSION 2: Drug Trials: Outcome Measures for Trials in Man
Chairperson: F. Gasparini, Novartis Pharma AG, Basel, Switzerland

S.W. Porges, University of Illinois, Chicago: The Polyvagal Theory: Insights into the selection of outcome measures.
J.T. McCracken, University of California, Los Angeles Neuropsychiatric Institute: Lessons from drug therapy of autism for Fragile X: Measurement challenges.
I. Boutet, University of Toronto, Scarborough, Canada: Novel behavioral tests to evaluate treatment outcome in Fragile-X syndrome.
E. Berry-Kravis, Rush Children's Hospital, Chicago, Illinois: Safety and efficacy of ampakine CX516 in Fragile-X syndrome.
R.J. Hagerman, University of California, Davis Health System, Sacramento: A multicenter trail of lithium for treatment of Fragile-X syndrome.

SESSION 3: Biology and Regulation of FMRP/FMR1: Phenotype Emanating from Molecular Studies
Chairperson: S.T. Warren, Emory University School of Medicine, Atlanta, Georgia

J. Darnell, The Rockefeller University, New York: RNA targets of the KH2 domain of FMRP and their role in translation.
J.R. Fallon, Brown University, Providence, Rhode Island: Regulation of *Fmr1* gene expression.
E. Klann, Baylor College of Medicine, Houston, Texas: Alteration in protein expression in *Fmr1* knockout mice.
I.J. Weiler, University of Illinois, Urbana-Champaign: Translational pathways: A diagnostic tool?

SESSION 4: mGluR Regulation at the Synapse in FXS
Chairperson: W.T. Greenough, University of Illinois, Urbana

M. Hayashi, Massachusetts Institute of Technology, Cambridge: An interaction between FMRP and p21-activated kinase (PAK).

M.F. Bear, HHMI/Massachusetts Institute of Technology, Cambridge: The mGluR theory of Fragile X.

R.P. Bauchwitz, Columbia University, New York: Further evidence for involvement of mGluR signaling in Fragile-X syndrome.

R. Denman, New York State Institute for Basic Research, Staten Island: FMRP: A regulator of mGluR5 mRNA?

K. Huber, University of Texas Southwestern Medical Center, Dallas: Role of FMRP in synaptic transmission and plasticity.

G.J. Bassell, Albert Einstein College, Bronx, New York: Metabotropic glutamate receptor regulation of FMRP trafficking and morphological plasticity.

P.W. Vanderklish, Scripps Research Institute, La Jolla, California: Regulatory interactions between synaptic structure and local translation.

R.K.S. Wong, State University of New York–Health Science Center, Brooklyn: Signaling pathway for neuronal plasticity in mGluR-induced epileptogenesis.

SESSION 5: Potential Drug Targets and Treatment Strategies
Chairperson: E. Berry-Kravis, Rush Children's Hospital, Chicago, Illinois

T.A. Jongens, University of Pennsylvania School of Medicine, Philadelphia: Pharmacological rescue of the *Drosophila* Fragile-X model.

W. Spooren, F. Hoffmann–La Roche, Basel, Switzerland: A comparison of the effects of MPEP and diazepam in rodent models of anxiety and cognition.

M. Toth, Cornell University Medical College, New York: GABA(B) receptor hypersensitivity and reversal of the Fragile-X phenotype by baclofen in mice.

J. Lauterborn, University of California, Irvine: AMPA receptor up-modulators and mental retardation in Fragile X.

F. Gasparini, Novartis Pharma AG, Basel, Switzerland: Identification and profiling of PET imaging agents for the mGlu5 receptor.

K. Shiosaki, Sention, Inc., Providence, Rhode Island: Development of mGluR receptor antagonist for Fragile X.

General Discussion/Wrap Up and Strategy

Spinal Muscular Atrophy: Neuronal Rescue and Repair From Laboratory to Clinic

March 13–16

FUNDED BY **Spinal Muscular Atrophy Foundation**

ARRANGED BY **K.E. Davies**, University of Oxford
L. Eng, Spinal Muscular Atrophy Foundation
T.M. Jessell, Columbia University
C. Joyce, Spinal Muscular Atrophy Foundation
A.E. MacKenzie, Children's Hospital of Eastern Ontario

Welcome and Introduction of Speaker: T.M. Jessell, Columbia University, New York
D.C. De Vivo, The Neurological Institute, Columbia University Medical Center, New York: Discussion of clinical manifestations of SMA and implications for therapeutic development.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
D. Singh, Spinal Muscular Atrophy Foundation, New York

SESSION 1: Biochemistry of SMN
Chairperson: A.E. MacKenzie, Children's Hospital of Eastern Ontario, Canada: Introduction of session topic.

G. Dreyfuss, HHMI/University of Pennsylvania School of Medicine, Philadelphia: The SMN complex.
J.L. Manley, Columbia University, New York: Mechanism of SMN2 exon 7 splicing inhibition.
A. Krainer, Cold Spring Harbor Laboratory: SMN2 splicing as a target.

General Discussion: B. Wirth, University of Cologne, Germany
G.J. Bassell, Albert Einstein College, Bronx, New York
D.W. Cleveland, University of California, San Diego, La Jolla

Key Points
Summary: A.E. MacKenzie, Children's Hospital of Eastern Ontario, Canada

SESSION 2: Druggable Targets Evolving from SMA Biology
Chairperson: S.C. Landis, NINDS/National Institutes of Health, Bethesda, Maryland: Introduction of session topic.

L.L. Rubin, Curis, Inc., Cambridge, Massachusetts: Motor-neuron-based screening for small molecules that increase SMN levels.
B.R. Stockwell, Columbia University, New York: Diagramming disease networks with chemical and biological tools.
A. Sands, Lexicon Genetics, The Woodlands, Texas: Mining the druggable genome for SMA targets.

General Discussion: G. O'Neill, Biogen IDEC, Cambridge, Massachusetts
C. Keith, CombinatoRx Inc., Boston, Massachusetts
K.W. Klinger, Genzyme Genetics Inc., Framingham, Massachusetts
D.W. Cleveland, University of California, San Diego

Key Points
Summary: D.W. Cleveland, University of California, San Diego, La Jolla

A. Krainer, D. DeVivo

SESSION 3: Cell Biology and Motor Neuron Function
Chairperson: K.H. Fischbeck, NINDS/National Institutes of Health, Bethesda, Maryland: Introduction of session topic.

G.J. Bassell, Albert Einstein College, Bronx, New York: Transport of an SMN-Gemin complex in neurons.
M. Sendtner, Universitat Wuerzburg, Germany: Functional and morphological alterations in isolated motor neurons from SMN-deficient mice.
E.M.C. Fisher, National Hospital for Neurology and Neurosurgery, London, United Kingdom: Crossing the Loa Dynein mutant mouse to a sod transgenic that models motor neuron diseases/ALS.

General Discussion: Z. He, Children's Hospital, Boston, Massachusetts
A.J. Tobin, MRSSI/High Q Foundation, New York
G. Davis, University of California, San Francisco

Key Points
Summary: C. Henderson, Institute of Marseille, Universite de la Mediterranee, France

SESSION 4: Topic A: Model Organisms and SMA Genetics
Chairperson: K.E. Davies, University of Oxford, United Kingdom: Introduction of session topic.

W. Thompson, University of Texas, Austin: Why Schwann cells are of interest in the pathology of SMA.
G. Davis, University of California, San Francisco: Identification of new mutations that cause synapse retraction and new mutations that prevent synapse retraction.
M. van den Heuvel, University of Oxford, United Kingdom: Use of *Drosophila melanogaster* as a model for SMA function.

General Discussion: B. McCabe, Columbia University, New York
A.H. Burghes, Ohio State University, Columbus
U.R. Monani, Columbia University, New York
J. Melki, INSERM, Evry, France

SESSION 5: Topic B: Therapeutic Implications Evolving from Genetics and Cell Biology
Chairperson: K.E. Davies, University of Oxford, United Kingdom: Introduction of session topic.

A.H.M. Burghes, Ohio State University, Columbus: Is SMN2 a good target for therapy in spinal muscular atrophy?
D.W. Cleveland, University of California, San Diego, La Jolla: A molecular therapy for familial ALS.
Z. He, Children's Hospital, Boston, Massachusetts: Axon regeneration and SMA.

General Discussion: R. Pacifici, MRSSI Inc., New York
J. Jareck, Families of SMA, Libertyville, Illinois
J. Melki, INSERM, Evry, France

Key Points
Summary: K.E. Davies, University of Oxford, United Kingdom

SESSION 6: Summary
Chairperson: T.M. Jessell, Columbia University, New York

Discussants: A.E. MacKenzie, Children's Hospital of Eastern Ontario, Ottawa, Canada
C. Henderson, Universite de la Mediterranee, Marseille, France
K.E. Davies, University of Oxford, United Kingdom
D.W. Cleveland, University of California, San Diego, La Jolla.

Discussion/Next Steps

A Critical Review of Melanoma: Genomic Approaches with Therapeutic Promise

March 20–23

FUNDED BY Ann L. and Herbert J. Siegel Fund of the Jewish Communal Fund; Melanoma Research Foundation; Chiron Corporation; Agencourt Bioscience Corporation; PICO Atlantic

ARRANGED BY **D. Becker**, University of Pittsburgh
M. McMahon, University of California

Introduction and Overview of Meeting: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory
D. Becker, University of Pittsburgh, Pennsylvania
M. McMahon, University of California, San Francisco

SESSION 1: Genomic Strategies to Identify Novel Genes in Melanoma and Nevi I
Chairperson: D. Pinkel, University of California, San Francisco

R. Lucito, Cold Spring Harbor Laboratory: ROMA, CNPs, and cancer.
P. Lichter, DKFZ, Heidelberg, Germany: Matrix-based CGH for tumor progression and diagnostics.
B. Bastian, University of California, San Francisco: Genetic classification of melanoma.
R. Halaban, Yale University School of Medicine, New Haven, Connecticut: Differential gene expression analysis revealing new pathways, associations, and directions for melanoma.

General Discussion and Key Points of Session

SESSION 2: Genomic Strategies to Identify Novel Genes in Melanomas and Nevi II
Chairperson: D. Becker, University of Pittsburgh, Pennsylvania

D. Becker, University of Pittsburgh, Pennsylvania: Analysis of genes identified in melanoma and nevus SAGE libraries and by microarrays.
D.E. Elder, Hospital of University of Pennsylvania, Philadelphia: Gene expression profiling in melanocytic lesions.
F. Marincola, National Institutes of Health, Bethesda, Maryland: cDNA arrays and melanoma immune responsiveness.
S. Hewitt, National Cancer Institute, Bethesda, Maryland: Tissue microarrays in translational research.
T. Kapoor, The Rockefeller University, New York: Chemical genetic analysis of chromosome segregation.
M. McManus, University of California, San Francisco: Mammalian RNA interference pathways from the perspective of a mouse.

General Discussion and Key Points of Session

SESSION 3: Melanoma Markers, Protein Targets, and Optical Imaging
Chairperson: D.E. Elder, Hospital of University of Pennsylvania, Philadelphia

N. Gruis, Leiden University Medical Centre, The Netherlands: Tumor markers in uveal melanoma identified by gene expression profiling.
G.P. Nolan, Stanford University School of Medicine, California: Potentiated single-cell cancer proteomics define patient network response to therapy.
A. Gudkov, Lerner Research Institute, Cleveland, Ohio: Why melanomas frequently maintain wild-type p53.
S. Simon, The Rockefeller University, New York: Tracking metastatic tumor cell extravasation by opitcal imaging and application of quantum dots.
I.J. Bigio, Boston University, Massachusetts: Optical scattering spectroscopy to noninvasively distinguish nevi.

General Discussion and Key Points of Session

SESSION 4: Gene Targeting and Analysis of Melanoma
Chairperson: M. McMahon, University of California, San Francisco

E. Dupin, CNRS, Nogent-sur-Marne, France: Neural crest stem cells in the development and maintenance of pigment cells.
S. Johnson, University of Washington School of Medicine, St. Louis, Missouri: Melanocyte stem cells in the zebra fish.
D.E. Fisher, Dana-Farber Cancer Institute, Boston, Massachusetts: Regulation of the melanoma prognostic marker, melastatin, in melanocytes and melanoma.
M. McMahon, University of California, San Francisco: Oncogenic transformation of mammalian cells by BRAF.
C.R. Goding, Marie Curie Research Institute, Surrey, United Kingdom: The Brn2-BRAF connection in melanoma.
L. Schuchter, University of Pennsylvania, Philadelphia: Melanoma therapy: High-dose, low-dose, no dose, which dose?

General Discussion and Key Points of Session

SESSION 5: Insights into Melanoma Gene Regulation
Chairperson: G. Merlino, National Cancer Institute, Bethesda, Maryland

G. Merlino, National Cancer Institute, Bethesda, Maryland: UV induction of melanoma: What the mouse can tell us.
Z.A. Ronai, The Burnham Institute, La Jolla, California: Transcription factors as targets for melanoma therapy.
B. Felding-Habermann, The Scripps Research Institute, La Jolla, California: Targeting adhesive mechanisms in melanoma metastasis.
M. Bar-Eli, M.D. Anderson Cancer Center, Houston, Texas: AP-2 expression in melanoma TMAs: Inverse correlation with progression.
J. Dong, Mount Sinai Medical Center, New York: Effects on proliferation and melanogenesis by inhibition of mutant BRAF and expression of wild-type INK4A in melanoma cells.

Future Avenues and Goals of Melanoma Research

R. Halaban, M. Bar-Eli

History of Science: Archives and Oral History

April 3–5

FUNDED BY **The Rockefeller Archive Center and Cold Spring Harbor Laboratory**

ARRANGED BY **M. Pollock,** Cold Spring Harbor Laboratory
D.H. Stapleton, The Rockefeller Archive Center

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
M. Pollock, Cold Spring Harbor Laboratory
D.H. Stapleton, The Rockefeller Archive Center, Sleepy Hollow, New York

SESSION 1: Owning the Past, Serving the Future
Chairperson: L.R. Hiltzik, The Rockefeller Archive Center, Sleepy Hollow, New York

J. Sheppard, Wellcome Library for the History and Understanding of Medicine, London, United Kingdom: The transition of ownership: Players and stakes.
P.B. Hirtle, Cornell University, Ithaca, New York: Copyright ownership in scientific archives.
S.S. Hodson, The Huntington Library, San Marino, California: Secrets revealed or sealed: Privacy in collections of personal papers.
T.J. Connors, University of Maryland, College Park: Scientific information in the federal government—The emerging partisan divide: A citizen-archivist's impressions.

J. Sheppard, R. Olby

SESSION 2: Coping with the Digital Era in Scientific Research
Chairperson: R. Burian, Virginia Polytechnic Institute and State University, Blacksburg

T. Rosko, Massachusetts Institute of Technology, Cambridge: Challenges of collecting and preserving scientific records in the digital age.
D.H. Stapleton, The Rockefeller Archive Center, Sleepy Hollow, New York: Just doing it: The Smithsonian Institution Archives–Rockefeller Archive Center Collaborative Electronic Records Project.
R. Moore, University of California, San Diego, La Jolla: Preservation of scientific collections using data grid technology.

SESSION 3: Different Perspectives: Historians, Scientists, and Institutions Views of Archives
Chairperson: M. Sniffin-Marinoff, Harvard University, Cambridge, Massachusetts

M.L. Levitt, American Philosophical Society Library, Philadelphia, Pennsylvania: Primary sources: Expectations of historians vs. operational realities in archives.
S. de Chadarevian, Max-Planck Institute for the History of Science, Berlin, Germany: A historian's experience working on current science.
R.C. Olby, University of Pittsburgh, Pennsylvania: A biographer's hopes and the subject's expectations.
P.J. Wosh, New York University, New York: Institutional perspectives.
C.S. Mead, Oregon State University, Corvallis: Digital collections with narrative: Expanding our constituencies.
M. Pollock, Cold Spring Harbor Laboratory/D.H. Stapleton, The Rockefeller Archive Center, Sleepy Hollow, New York: General discussion.

SESSION 4: Oral History and Science
Chairperson: N.C. Comfort, Johns Hopkins Medical Institutions, Baltimore, Maryland

V. Dawson, History Enterprises, Inc., Cleveland, Ohio: Oral history as investigative tool.

R.E. Doel, Oregon State University, Corvallis: Voices in a discordant chorus: Oral history and the recent history of scientific institutions.

M. Pollock, Cold Spring Harbor Laboratory: Recording science and life through (oral) autobiography.

E.M. Tansey, Wellcome Trust Centre for the History of Medicine, London, United Kingdom: Who is oral history for? Reflections on witness seminars in modern biomedicine.

SESSION 5: Discussion on Major Issues of the Meeting

D.H. Stapleton, The Rockefeller Archive Center, Sleepy Hollow, New York
P.B. Hirtle, Cornell University, Ithaca, New York

Key Points

R. Burian, W. Summers, B. Stillman

Polyploidy, Heterosis, and Genomic Balance

April 10–13

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J.A. Birchler**, University of Missouri
L. Comai, University of Washington

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
J.A. Birchler, University of Missouri, Columbia
L. Comai, University of Washington, Seattle

SESSION 1
Chairperson: J.A. Birchler, University of Missouri, Columbia

J. Silverthorne, The National Science Foundation, Arlington, Virginia: Polyploidy, heterosis, and genomic balance: The NSF perspective.

G.C. Gibson, North Carolina State University, Raleigh: Estimating heritability and nonadditivity in fly and human expression profiles.

C. Disteche, University of Washington, Seattle: The complex regulation of the mammalian X chromosome: Up-regulation and inactivation.

B. Oliver, NIDDK/National Institutes of Health, Bethesda, Maryland: X-chromosome dosage compensation in the *Drosophila* germ line.

R.H. Reeves, The Johns Hopkins University, Baltimore, Maryland: The DSCR is not critical for Down's syndrome.

B. Dilkes, D. Soltis, J. Wendel, K. Adams

SESSION 2
Chairperson: R.W. Doerge, Purdue University, West Lafayette, Indiana

D.N. Duvick, Iowa State University, Ames: Contributions of heterosis to maize yield, plant height, and maturity, during six decades of breeding.
D. Zamir, Hebrew University of Jerusalem, Rehovot, Israel: Heterotic QTLs in tomato are limited to traits associated with reproductive success.
D. Jackson, Cold Spring Harbor Laboratory: Candidate genes for quantitative traits underlying inflorescence architecture in maize.
J.F. Wendel, Iowa State University, Ames: Genome evolution in polyploid cotton.
K. Adams, University of British Columbia, Vancouver, Canada: Organ-specific gene silencing in polyploids and hybrids.

SESSION 3
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

T.C. Osborn, University of Wisconsin, Madison: Phenotypic effects of polyploidy-induced variation in homologous allele dosage.
L. Comai, University of Washington, Seattle: Polyploidy and dosage in plants: A matter of chromatin regulation?
R. Scott, University of Bath, United Kingdom: Genomic balance in *Arabidopsis thaliana* and its relatives.
B. Dilkes, University of Washington, Seattle: Dosage effects in interploidy crosses of *Arabidopsis thaliana*.
O. Mittelsten Scheid, Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria: Formation of epialleles: Their maintenance and interaction in polyploid *Arabidopsis*.
C.S. Pikaard, Washington University, St. Louis, Missouri: Chromosomal rearrangements and gene-silencing events affecting NORs and rRNA genes in *suecica*.

SESSION 4
Chairperson: T. C. Osborn, University of Wisconsin, Madison

J.C. Pires, University of Wisconsin, Madison: Novel genotypic and phenotypic changes among resynthesized Brassica allopolyploids.
B. Chalhoub, INRA/CNRS-URGV, France: Molecular basis of polyploidy-related gene loss in wheat species (*Triticum* and *Aegilops*).
A.A. Levy, Weizmann Institute of Science, Rehovot, Israel: Bread as a model system to study polyploidy and interspecific hybrids.
A. Paterson, University of Georgia, Athens: Polyploidy and angiosperm comparative genomics.
D.E. Soltis, University of Florida, Gainesville: Genetic and genomic consequences of recent and recurring allopolyploidy in *Tragopogon* (Asteracease).

SESSION 5
Chairperson: L. Comai, University of Washington, Seattle

R.W. Doerge, Purdue University, West Lafayette, Indiana: Locating QTL in polyploids: The things to think about... .
Z.J. Chen, Texas A&M University, College Station: Transcriptome divergence and mechanisms of nonadditive gene regulation in *Arabidopsis*.
R. Martienssen, Cold Spring Harbor Laboratory: Epigenomic variation.
J.A. Birchler, University of Missouri, Columbia: Biological implications of regulatory gene balance with special reference to studies in maize and *Drosophila*.

General Discussion/Key Points of Meeting

Neurobiology of Decision-making

May 22–25

FUNDED BY **The Swartz Foundation**

ARRANGED BY **C. Brody**, Cold Spring Harbor Laboratory
M.N. Shadlen, HHMI/University of Washington
X.-J. Wang, Brandeis University

Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: M.N. Shadlen, HHMI/University of Washington, Seattle

R. Gallistel, Rutgers University, New Brunswick, Piscataway, New Jersey: The irrelevance of the law of effect in unconstrained free choice with random rewards.
D. Lee, University of Rochester, New York: Computation of values in the primate frontal cortex.
P.W. Glimcher, New York University, New York: Physiological and economic models of decision-making.
W. Schultz, University of Cambridge, United Kingdom: Reward responses as potential input signals for decision-making.
P.R. Montague, Baylor College of Medicine, Houston, Texas: Neural correlates of policy adjustment in a dynamic economic game.

SESSION 2
Chairperson: C. Brody, Cold Spring Harbor Laboratory

H.R. Heekeren, Humboldt University, Berlin, Germany: A general mechanism for perceptual decision-making in the human brain.
M.N. Shadlen, HHMI/University of Washington, Seattle: How does the brain combine evidence with prior probability?
X.-J. Wang, Brandies University, Waltham, Massachusetts: A neuronal microcircuit model for reaction time behavior.
R. Ratcliff, Ohio State University, Columbus: An analysis of the effects of aging in two choice tasks using sequential sampling models.
J.D. Schall, Vanderbilt University, Nashville, Tennessee: Choice, decision, and action investigated with visually guided saccades.

SESSION 3
Chairperson: G.D. Logan, Vanderbilt University, Nashville, Tennessee

J. Gold, University of Pennsylvania, Philadelphia: Multiple roles of experience in neural circuits that form perceptual decisions.
P. Holmes, Princeton University, New Jersey: What's optimal about decision-making for two and more choices?
K. Krug, University Laboratory of Physiology, Oxford, United Kingdom: Controlled intervention in perceptual decision-making.
P.L. Smith, The University of Melbourne, Victoria, Australia: An integrated model of decision-making and visual attention.
S. Deneve, Institut des Sciences Cognitives, Bron, France: Explicit neural space and implicit probability space.

P. Glimcher, D. Lee

SESSION 4
Chairperson: X.-J. Wang, Brandeis University, Waltham, Massachusetts

Z.F. Mainen, Cold Spring Harbor Laboratory: Neural circuits underlying olfactory decisions in the rat.
O. Hikosaka, National Eye Institute, Bethesda, Maryland: Basal ganglia mechanisms of reward-oriented eye movement.
M. Basso, University of Wisconsin, Madison: Basal ganglia microstimulation affects memory and movement.
A. Koulakov, Cold Spring Harbor Laboratory: How to define command neurons?
W.B. Kristan, University of California, San Diego: The dynamics of decision-making by leech neurons.

SESSION 5
Chairperson: J.D. Schall, Vanderbilt University, Nashville, Tennessee

J.D. Cohen, Princeton University, New Jersey: Role of locus coeruleus in adaptive adjustments of gain and optimal performance in simple decision-making tasks.
C. Brody, Cold Spring Harbor Laboratory: Combining working memory and decision-making in a simple neural model of prefrontal cortex.
M. Platt, Duke University Medical Center, Durham, North Carolina: Neural mechanisms of social decision-making.
A. Kacelnik, Oxford University, United Kingdom: Decision-making under risk: Biological perspectives.
S. Makeig, Swartz Center for Computational Neuroscience, University of California, San Diego, La Jolla: Decisions have consequences.

General Discussion/Key Points of Meeting

Stem Cells and Axonal Regeneration: Strategies for the Treatment of ALS

September 11–14

FUNDED BY **The ALS Association**

ARRANGED BY **L. Bruijn**, The ALS Association
S.M. Strittmatter, Yale University
C.N. Svendsen, University of Wisconsin

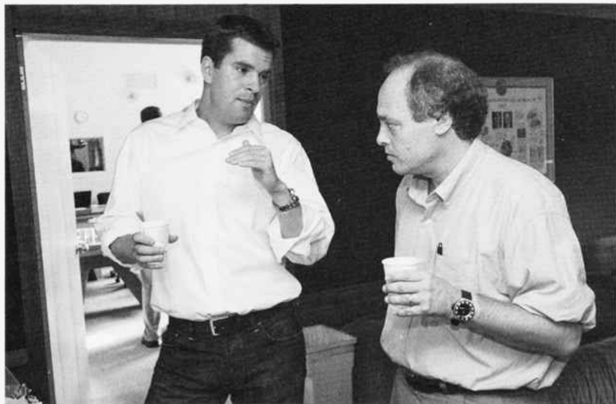
Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory
L. Bruijn, The ALS Association, Palm Harbor, Florida

SESSION 1: Introductory Session
Chairperson: L. Bruijn, The ALS Association, Palm Harbor, Florida

S.L. Pfaff, Salk Institute, La Jolla, California: Overview of motor neuron development and cell specification and cell fate.
S.A. Goldman, University of Rochester Medical Center, New York: Overview of stem cell biology.
S.M. Strittmatter, Yale University School of Medicine, New Haven, Connecticut: Overview of regeneration, axonal guidance cues, and inhibitory molecules.

SESSION 2: Neural Stem Cells
Chairperson: C.N. Svendsen, University of Wisconsin, Madison

P. Horner, University of Washington, Seattle: Endogenous stem cells in spinal cord and repair.
H. Wichterle, Columbia University, New York: Control of embryonic stem-cell-derived motor neuron subtype identity.
J.D. Macklis, Harvard Medical School, Massachusetts General Hospital, Boston: Molecular cues for upper motor neuron development.
A.R. Kriegstein, University of California, San Francisco: Neural stem and progenitor cells in the embryonic brain.
M.V. Sofroniew, University of California, Los Angeles: Are astrocytes stem cells?
N. Gaiano, Johns Hopkins University Medical School, Baltimore, Maryland: Embryonic neural stem cell heterogeneity.



K. Eggan, S. Goldman

SESSION 3: Axonal Regeneration
Chairperson: S.M. Strittmatter, Yale University School of Medicine, New Haven, Connecticut

M. Filbin, Hunter College of CUNY, New York: Myelin inhibitors of regeneration: How they work and how to overcome them.

J. Lichtman, Harvard University, Cambridge, Massachusetts: Monitoring growth and retraction of axons in situ.

SESSION 4: Stem Cells and Neurodegenerative Diseases
Chairperson: M. Swash, Royal London Hospital, United Kingdom

C.N. Svendsen, University of Wisconsin, Madison: Overview of stem cells and neurodegenerative diseases: Lessons for ALS from PD.

S.-C. Zhang, University of Wisconsin, Madison: Generation of human motor neurons: Applications for ALS studies.

P. Aebischer, Integrative Bioscience Institute, Swiss Federal Institute of Technology, Lausanne, Switzerland: Lentivirus-mediated gene silencing of SOD-1 for the treatment of ALS.

SESSION 5: Axonal Regeneration
Chairperson: M. Filbin, Hunter College of CUNY, New York

B.A. Barres, Stanford Medical School, California: Why is Wallerian degeneration so slow in the CNS?

J.D. Milbrandt, Washington University, St. Louis, Missouri: Wallerian degeneration, neurotrophins, and axonal growth.

SESSION 6: Application of Stem Cell Technologies in Understanding Disease
Chairperson: S.A. Goldman, University of Rochester Medical Center, New York

I. Wilmut, Roslin BioCentre, Midlothian, United Kingdom: Cells from cloned human embryos in studies of ALS.

K. Eggan, Harvard University, Cambridge, Massachusetts: Cloning and stem cells: Building cell-based models of ALS.

L. Goldstein, HHMI/University of California, San Diego School of Medicine, La Jolla: Identifying cells to be replaced in ALS.

Y. Zou, University of Chicago, Illinois: Guidance of axons along the rostral-caudal axis of the spinal cord.

SESSION 7: Stem Cells and Neurodegenerative Diseases
Chairperson: T. Miller, University of California, San Diego

M. Swash, Royal London Hospital, United Kingdom: Overview of clinical challenges in ALS.

L.J. Martin, The Johns Hopkins University, Baltimore, Maryland: Adult stem cells and ALS models.

D. Kerr, The Johns Hopkins University, Baltimore, Maryland: Overview of current efforts in model systems for ALS.

N. Boulis, The Cleveland Clinic Foundation, Ohio: Neurosurgical challenges for invasive therapies for ALS.

O. Isacson, Harvard Medical School, Belmont, Massachusetts: Neuronal replacement therapy for neurodegeneration using stem cells.

General Discussion/Future Directions

Closing Remarks: R.V. Abendroth, Milwaukee, Wisconsin

From Markers to Models: Integrating Data to Make Sense of Biologic Systems

September 18–21

FUNDED BY **Centers for Disease Control & Prevention; CFIDS Association of America**

ARRANGED BY **S.D. Vernon**, Centers for Disease Control and Prevention
W.C. Reeves, Centers for Disease Control and Prevention

Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: W.C. Reeves, Centers for Disease Control and Prevention, Atlanta, Georgia

S.D. Vernon, Centers for Disease Control and Prevention, Atlanta, Georgia: C3: Progress toward the CFS interaction map.

S.M. Lin, Northwestern University, Chicago, Illinois: Lessons learned from 5 years of CAMDA.

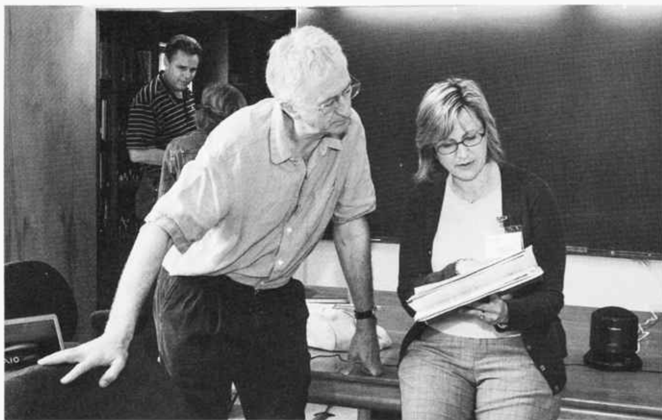
Team 1: Toward the Nosology of Chronic Unexplained Fatigue
1. Initial data perusal to direct hypothesis-driven analysis
2. Analytical approach(es)
3. Results and biological interpretation

B. Mishra, New York University: Computational and experimental framework to understand disease pathogenesis.

Team 2: CFS: From Constructs to Mechanisms
1. Initial data perusal to direct hypothesis-driven analysis
2. Analytical approach(es)
3. Results and biological interpretation

J. Shoemaker, Duke University Medical Center, Durham, North Carolina: Mathematical and statistical challenges for high-throughput data.

Team 3: Challenges of Elucidating Pathophysiology in Complex Disorders
1. Initial data perusal to direct hypothesis-driven analysis
2. Analytical approach(es)
3. Results and biological interpretation



P. White, K. McCleary

SESSION 2
Chairperson: A.H. Miller, Emory University School of Medicine, Atlanta, Georgia

M. Demitrack, Neuronetics, Inc., Malvern, Pennsylvania: Clinical perspectives on therapeutic interventions for CFS.
J. DiStefano, University of California, Los Angeles: Dynamic systems modeling and thyroid hormone regulation and metabolism in mammals.

Team 4: Bridging the Gap between the Neuroendocrine and Immune Systems
1. Initial data perusal to direct hypothesis-driven analysis
2. Analytical approach(es)
3. Results and biological interpretation

SESSION 3: Breakout Session for Non-C3 Participants to Evaluate and Summarize Team Approach

B. Mishra, New York University/M. Demitrack, Neuronetics, Inc., Malvern, Pennsylvania: Report on Team 1.
J. Shoemaker, Duke University Medical Center, Durham, North Carolina: Report on Team 2.
S.M. Lin, Northwestern University, Chicago, Illinois/W.C. Reeves, Centers for Disease Control and Prevention, Atlanta, Georgia: Report on Team 3.
J. DiStefano, University of California, Los Angeles/A.H. Miller, Emory University School of Medicine, Atlanta, Georgia: Report on Team 4.

SESSION 4: Team Breakout Session: Next Steps for Each Team

- Regroup?
- Complete Analysis?
- Delegate?
- Publication(s) Plan?

Next Steps
- Computational and Biologic Validation
- Publications

Pathogenesis and Early Events in Viral Infection

September 25–28

FUNDED BY **U.S. Defense Department (through a grant to the Institute for Comparative Genomics)**

ARRANGED BY **R. Breeze**, Institute for Comparative Genomics
F. Horn, Institute for Comparative Genomics
W. Laegreid, USDA Agricultural Research Service
D.L. Rock, University of Illinois

Introduction and Charge for Meeting: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
R. Breeze, Institute for Comparative Genomics, Washington, D.C.

SESSION 1: Early Events in Virus Infection

J.A. Hiscox, University of Leeds, United Kingdom: The nucleolus as a gateway to virus infection.

G. Sutter, Paul-Ehrlich-Institut, Langen, Germany: Control of apoptosis and translation mark essential early checkpoints in the vaccinia virus MVA.

E. Tulman, University of Connecticut, Storrs: Comparative viral genomics for identification of host-range determinants and virulence factors.

SESSION 2: Host Resistance

A.A. Ashkar, McMaster University Health Science Center, Hamilton, Canada: Induction of innate antiviral immunity by TLR agonists/ligands.

A. Garcia-Sastre, Mount Sinai School of Medicine, New York: Reverse-genetics-derived influenza viruses.

H.W. Virgin, Washington University School of Medicine, St. Louis, Missouri: New approaches to defining mechanisms of virus resistance and pathogen discovery.

M. Brinton, Georgia State University, Atlanta: Comparison of the responses of MEFs from congenic flavivirus-resistant and -susceptible mice to West Nile virus.

A. Perelson, T. Endy, A. Garcia-Sastre

SESSION 3: Pathogenesis

T. Barrett, Institute for Animal Health, Surrey, United Kingdom: Molecular determinants of pathogenesis by rinderpest virus.

T.P. Endy, Walter Reed Army Institute of Research, Silver Spring, Maryland: Pathogenesis and early events in acute dengue virus infection.

J.N. MacLachlan, University of California, Davis: Role of vascular epithelium in selected animal virus infections: Villain or victim?

A. Alcami, Centro Nacional de Biotecnologia, Madrid, Spain: Immune modulation by cytokine receptors from variola and ectromelia viruses.

L. Hensley, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland: Temporal analysis of ebola and Marburg hemorrhagic fever in cynomologus macaques.

SESSION 4: Pathogenesis

E.S. Mocarski, Stanford University School of Medicine, California: Virus-mediated recruitment of host cells for systemic dissemination within the host.

C. Jones, University of Nebraska, Lincoln: Analysis of α-herpesvirus genes expressed in latently infected neurons.

E. Ivanovna Ryabchikova, State Research Center of Virology and Biotechnology, Koltsovo, Russia: Laboratory accident of ebola hemorrhagic fever: Diagnostics, clinical course, and treatment.

G. Keil, Friedrich-Loeffler Institut, Greitswald-Insel Riems, Germany: Engineering glycoprotein B of bovine herpesvirus 1 as transporter for biologically active secreted proteins.

SESSION V: Pathogenesis

A.S. Perelson, Los Alamos National Laboratory, New Mexico: Modeling the kinetics of acute virus infection.

J. Paragas, U.S. Army Medical Research Institute of Infectious Disease, Fort Detrick, Maryland: A bright light in biodefense for orthopox viruses.

R. Breeze, Institute for Comparative Genomics, Washington, D.C.: Summary and discussion.

Mitochondria in Neurological Disease and Aging

October 2–5

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M.F. Beal**, Weill Medical College of Cornell University
D.C. Wallace, University of California

Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory
M.F. Beal, Weill Medical College of Cornell University, New York
D.C. Wallace, University of California, Irvine

SESSION 1: Genetics and Pathophysiology
Chairperson: M.F. Beal, Weill Medical College of Cornell University, New York

G. Attardi, California Institute of Technology, Pasadena: Termination-factor-mediated DNA looping controls human mitochondrial rRNA synthesis.
J.A.M. Smeitink, Radbound University Medical Center, Nijmegan, The Netherlands: Cell biological consequences of human complex-1 deficiency.
B.M. Spiegelman, Dana-Farber Cancer Institute, Boston, Massachusetts: Regulation of mitochondria biogenesis and bioenergetics through the PGC-1 coactivators.
R.C. Scarpulla, Northwestern University Medical School, Chicago, Illinois: PGC-1-related coactivator (PRC): A potential link between cell proliferation and respiratory chain expression.

SESSION 2: Biochemistry and Physiology
Chairperson: B.N. Ames, University of California, Berkeley

R.A. Capaldi, University of Oregon, Eugene: Measurement of oxidative damage to OXPHOS proteins in neurodegeneration: Toward identification of biomarker for Alzheimer's and Parkinson's diseases.
P. Giuseppe Pelicci, European Institute of Oncology, Milan, Italy: Regulation of ROS metabolism by p66Shc.
P. Bernardi, University of Padova, Italy: The mitochondrial permeability transition in degenerative diseases and aging.

SESSION 3: Animal Models
Chairperson: B.M. Spiegelman, Dana-Farber Cancer Institute, Boston, Massachusetts

T. Prolla, University Wisconsin, Madison: Aging, oxidative stress, and apoptosis in mitochondrial mutator mice.
C.T. Moraes, University of Miami, Florida: A mouse model of COX deficiency in the CNS.
D. Walker, California Institute of Technology, Pasadena: Mitochondrial dysfunction in *Drosophila*.

SESSION 4: Brain Disease and Aging
Chairperson: D.C. Wallace, University of California, Irvine

M.F. Beal, Weill Medical College of Cornell University, New York: Therapeutic approaches to mitochondrial dysfunction in neurodegenerative diseases.
B.N. Ames, University of California, Berkeley, Oakland: Delaying (or accelerating) the mitochondrial decay of aging.
L.P. Guarente, Massachusetts Institute of Technology, Cambridge: Function of mitochondrial sirtuin Sirt4.

SESSION 5: Friedreich Ataxia, ALS, and AD
Chairperson: A.D. Roses, GlaxoSmithKline, Research Triangle Park, North Carolina

G. Isaya, Mayo Clinic, Rochester, Minnesota: Anti-oxidant functions of frataxin: Roles in Friedreich ataxia and other age-related conditions.
R.B. Wilson, University of Pennsylvania, Philadelphia: Mitochondrial dysfunction in Friedreich ataxia.
P. Pasinelli, Massachusetts General Hospital, Charlestown:

SOD1/BCl-2 complex: A role in the regulation of mitochondria cell death.

D.W. Cleveland, University of California, San Diego: Mitochondrial involvement in familial ALS.

G. Manfredi, Cornell University, New York: Mitochondrial involvement in SOD1–familial ALS.

A.D. Roses, GlaxoSmithKline, Research Triangle Park, North Carolina: An apoE4 isoform-specific mechanism for altered energy metabolism in Alzheimer's disease.

M. Ankarcrona, Karolinska Institutet, Huddinge, Sweden: Mechanisms of cell death in Alzheimer's disease: Focus on γ-secretase and mitochondria.

A. Kontush, Hopital de la Pitie, Paris, France: Amyloid-β peptide and mitochondria in Alzheimer's disease: Is there a link mediated by oxidative stress?

D.C. Wallace, University of California, Irvine: A mitochondrial paradigm for metabolic and degenerative diseases, cancer, and aging.

SESSION 6: Huntington's Disease and Parkinson's Disease
Chairperson: M.F. Beal, Weill Medical College of Cornell University, New York

G.V.W. Johnson, University of Alabama, Birmingham: Mutant Huntington compromises mitochondrial function.

R.L. Nussbaum, National Human Genome Research Institute, Bethesda, Maryland: Role of α-synuclein in lipid metabolism and mitochondrial function.

A. Abeliovich, Columbia University College of Physicians and Surgeons, New York: Oxidative stress and dopamine neuron survival: The role of DJ-1.

J.M. Vance, Duke University Medical Center, Durham, North Carolina: Mitochondria and late-onset Parkinson's disease.

M. Cookson, National Institutes of Health, Bethesda, Maryland: PINK1 and DJ-1, associated with recessive parkinsonism, delineate mitochondrial pathways important in neuronal survival.

Discussion:
Common Themes
Mitochondria
Neurological Diseases

Microbial Forensics 2005: Sample Management

October 16–19

FUNDED BY **U.S. Department of Homeland Security**

ARRANGED BY **S.E. Schutzer**, UMDNJ–New Jersey Medical School
B. Budowle, Federal Bureau of Investigation
J.P. Burans, U.S. Department of Homeland Security

Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory

Overview and Goals: B. Budowle, Federal Bureau of Investigation, Washington, D.C.

SESSION 1: Sampling/Collection Strategies I
Chairperson: B.L. Marrone, Los Alamos National Laboratory, New Mexico

D. Beecher, FBI Laboratory, Quantico, Virginia: Strategies for collection and sampling (known/unknown pathogen).

J. Fletcher, Oklahoma State University, Stillwater: Sampling issues for plant pathogens.

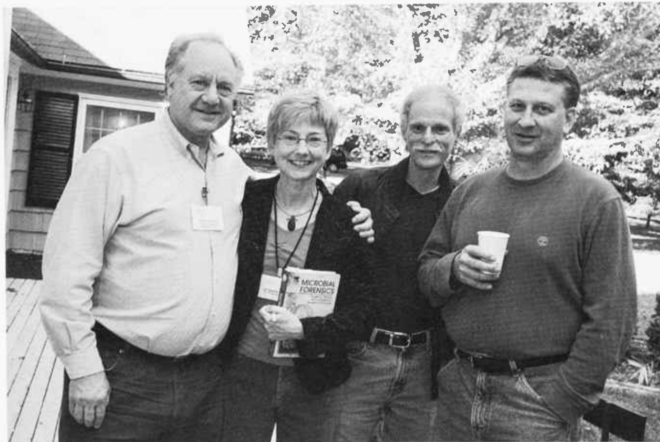
L.L. Rodriquez, ARS, USDA Plum Island Animal Disease Center, Greenport, New York: Sampling issues for animal pathogens.

J.E. LeClerc, Food and Drug Administration, Laurel, Maryland: Sampling issues for food pathogens.

SESSION 2: Sampling/Collection Strategies II
Chairperson: J.P. Burans, U.S. Department of Homeland Security, Frederick, Maryland

F.P. Keller, Federal Bureau of Investigation, Quantico, Virginia: FBI protocols for sample collection.

N. Valentine, Pacific Northwest National Laboratory, Richland, Washington: Protocols for collecting from substrates.



S. Schutzer, J. Fletcher, B. Budowle, M. Wilson

SESSION 3: Sampling/Collection Strategies III
Chairperson: B. Budowle, Federal Bureau of Investigation, Washington, D.C.

M. Hale, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland: Sampling issues/protocols for toxins.
T.G. Ksiazek, Centers for Disease Control and Prevention, Atlanta, Georgia: Sampling strategies for BSL3/4 agents.
L. Lindler, U.S. Department of Homeland Security, Frederick, Maryland: Sampling strategies for bacteria.
J.P. Burans, U.S. Department of Homeland Security, Frederick, Maryland: Sampling strategies and impact on downstream analyses.

SESSION 4: Sampling/Collection Strategies IV
Chairperson: P.T. Pesenti, U.S. Department of Homeland Security, Washington, D.C.

M.A. Heitkamp, Savannah River National Laboratory, Aiken, South Carolina: Collecting background controls.
B.L. Marrone, Los Alamos National Laboratory, New Mexico: The GAO report and beyond anthrax spores.

SESSION 5: Packaging, Shipping, and Storage (Integrity, Preservation, Safety, Regulations)
Chairperson: S.P. Velsko, Lawrence Livermore National Laboratory, California

W.T. Cobb, Cobb Consulting Services, Kennewick, Washington: Forensic plant issues.
Y.A. Lue, Quest Diagnostics Laboratories, Teterboro, New Jersey: Preservation of samples during shipping.
M. Hevey, NBFAC Research & Spoke Lab Program, Frederick, Maryland: Storage and preservation in the laboratory.

SESSION 6: Extraction/Preparation Strategies (Maintaining Integrity of Signatures) I
Chairperson: M.R. Wilson, FBI Academy, Quantico, Virginia

S.P. Velsko, Lawrence Livermore National Laboratory, California: Collection and preservation of samples for chemical and physical analysis.
K.L. Wahl, Pacific Northwest National Laboratory, Richland, Washington: Extraction strategies for proteins and other organic molecules.
C.R. Kuske, Los Alamos National Laboratory, New Mexico: Extraction strategies for nucleic acids.

SESSION 7: Extraction/Preparation Strategies (Maintaining Integrity of Signatures) II
Chairperson: S.E. Schutzer, UMDNJ–New Jersey Medical School, Newark

M.R. Wilson, FBI Academy, Quantico, Virginia: Nucleic acid concentration techniques.
J. Dunbar, Los Alamos National Laboratory, New Mexico: Removing or neutralizing inhibitors (of nucleic acid analyses).
M. Lipton, Pacific Northwest National Laboratory, Richland, Washington: Microbial extraction from dirt: Examples and complete proteomic analysis.

SESSION 8: Wrap-up Sessions
Chairperson: B. Budowle, Federal Bureau of Investigation, Washington, D.C.

M. Eshoo, Isis Pharmaceuticals, Carlsbad, California: Whole-genome amplification strategies and issues.
T. Cebula, U.S. Food and Drug Administration, Laurel, Maryland: Collating methods, value, and preparedness.
P.T. Pesenti, U.S. Department of Homeland Security, Washington, D.C.: New technologies on the horizon.
B.L. Marrone, Los Alamos National Laboratory, New Mexico: Research needs.

SESSION 9: Summary and Review
Chairperson: S.E. Schutzer, UMDNJ–New Jersey Medical School, Newark

B. Budowle, Federal Bureau of Investigation, Washington, D.C./ S.E. Schutzer, UMDNJ–New Jersey Medical School, Newark: Review of meeting and strategies for addressing gaps.

Introduction and Sustainable Use of Vaccines in Developing Countries

October 19–21

FUNDED BY **Albert B. Sabin Vaccine Institute, with the support from the Bill & Melinda Gates Foundation**

ARRANGED BY **K. Reilly**, Sabin Vaccine Institute
F.F. Songane, Ministerio da Saude

Welcome and Introduction of Speaker: **H.R. Shepherd**, Albert B. Sabin Vaccine Institute, New Canaan, Connecticut
G. Alleyne, Pan American Health Organization. Washington, D.C.: Immunization for all: A condition for health and development.
K. Reilly, Sabin Vaccine Institute, Rosemont, Pennsylvania and **F.F. Songane**, Ministerio da Saude, Maputo, Mozambique: Charge to the Conference.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

R.A. MacDougall, Albert B. Sabin Vaccine Institute, Washington, D.C.: Review of Delphi Survey

SESSION 1: Review of Current Immunization Status in Developing Countries
Moderators: K. Reilly, Sabin Vaccine Institute, Rosemont, Pennsylvania, and **F.F. Songane**, Ministerio da Saude, Maputo, Mozambique

Panel
J.-M. Okwo Bele, World Health Organization, Geneva, Switzerland
N. Antwi Agyei, Ghana Health Service, Accra
N. Van Cuong, National Expanded Programme on Immunisation (EPI), Hanoi, Vietnam

Open Discussion and Consensus
What lessons can be learned from the current situation?

SESSION 2: Financial Resources to Support Immunization: What Is Available, What Is Needed?
Moderators: K. Reilly, Sabin Vaccine Institute, Rosemont, Pennsylvania, and **F.F. Songane**, Ministerio da Saude, Maputo, Mozambique

Panel

J. Lob-Levyt, UNICEF, Geneva, Switzerland
I. Makumbi, Ministry of Health, Uganda
R. Levine, Center for Global Development, Washington, D.C.
G. Lamb, World Bank, Washington, D.C.
M. Harvey, USAID, Washington, D.C.

SESSION 3: Procurement and Supply of Existing and New Vaccines
Moderators: K. Reilly, Sabin Vaccine Institute, Rosemont, Pennsylvania, and **F.F. Songane,** Ministerio da Saude, Maputo, Mozambique

Panel

S. Jarrett, UNICEF, New York
J.K. Andrus, Pan American Health Organization, Washington, D.C.
F. Valente, Ministry of Health, Angola
W. Vandermissen, GlaxoSmithKline Biologicals, Rixensart, Belgium

SESSION 4: Review of Discussions and Consensus on Key Points
Moderators: K. Reilly, Sabin Vaccine Institute, Rosemont, Pennsylvania, and **F.F. Songane**, Ministerio da Saude, Maputo, Mozambique

SESSION 5: Opportunities and Recommendations for Sustainable Performance Levels in Immunization
Moderators: K. Reilly, Sabin Vaccine Institute, Rosemont, Pennsylvania, and **F.F. Songane**, Ministerio da Saude, Maputo, Mozambique

Panel

T. Belaye, Federal Democratic Republic of Ethiopia, Addis Ababa
G. Aslanyan, Canadian International Development Agency, Quebec, Canada
H. Sunman, Department of International Development, London, United Kingdom
A. Mahmoud, Merck & Co., Inc., Whitehouse Station, New Jersey
J.-M. Okwo-Bele, World Health Organization, Geneva, Switzerland

Conference Wrap Up/Next Steps

L.A. Miller, WentzMiller & Associates, Darien, Connecticut
K. Reilly, Sabin Vaccine Institute, Rosemont, Pennsylvania
F. Songane, Ministerio da Saude, Maputo, Mozambique

M. Kitambi, M. Harvey, T. Belaye

Epilepsy Genetics and Pharmacogenetics

October 23–26

FUNDED BY **UCB Pharma and the National Society for Epilepsy**

ARRANGED BY **N. Delanty**, Royal College of Surgeons in Ireland
D.B. Goldstein, Duke Institute for Genome Sciences and Policy
L. Sander, University College, London
S.M. Sisodiya, University College, London

Introduction: **J.A. Witkowski**, Banbury Center, Cold Spring Harbor Laboratory
D.B. Goldstein, Duke Institute for Genome Sciences and Policy, Durham, North Carolina

SESSION 1: Epilepsy: Epidemiological and Clinical Context
Chairperson: L. Sander, University College London, United Kingdom

S.M. Sisodiya, University College London, United Kingdom: Epilepsy: Setting the stage.
L. Sander, University College London, United Kingdom: Epidemiological considerations.
A. Lascelles, National Society for Epilepsy, London, United Kingdom: Perspectives from patients and family.

SESSION 2: Phenotyping and Genotyping: Issues
Chairperson: S.M. Sisodiya, University College London, United Kingdom

N. Delanty, Royal College of Surgeons in Ireland, Dublin: The promise and perils of epilepsy phenotyping?
J. Mulley, Women's and Children's Hospital, North Adelaide, Australia: The genetics of "simple" and complex idiopathic epilepsies.
D.H. Lowenstein, University of California, San Francisco: Large-scale phenotyping of epilepsy.

L. Sander, D. Goldstein, S. Sisodyia

SESSION 3: Epilepsy Genetics I
Chairperson: L. Sander, University College London, United Kingdom

M. Weale, University College London, United Kingdom: Representing genetic variation in a large-scale epilepsy genetic association study.
J. Hirschhorn, Children's Hospital, Boston, Massachusetts: Performing and interpreting association studies for complex traits.
J.O. McNamara, Duke University Medical Center, Durham, North Carolina: Genetic and molecular mechanisms of epileptogenetics.
J.L. Noebels, Baylor College of Medicine, Houston, Texas: Large-scale resequencing of ion channels in epilepsy: The Human Channelopathy Project.
D.L. Burgess, Baylor College of Medicine, Houston, Texas: Large-scale resequencing of ion channels in epilepsy: Design, implementation, and progress.
P.B. Crino, University of Pennsylvania Medical Center, Philadelphia: Somatic mutations during brain development that lead to sporadic brain malformations associated with epilepsy.

SESSION 4: Epilepsy Genetics II
Chairperson: N. Delanty, Royal College of Surgeons in Ireland, Dublin

R. Ottman, Columbia University, New York: Genetic epidemiology of the epilepsies.
O. Chiba-Falek, Human Genome Research Institute, NIH, Bethesda, Maryland: Regulation of α-synuclein (SNCA) expression in Parkinson's disease: Identification of regulatory polymorphisms.
G. Cavalleri, University College London, United Kingdom: BRD2 as a risk factor for JME.
C. Depondt, Universite Libre de Bruxelles, Belgium: Role of SCNIA in sporadic epilepsy and epilepsy pharmacogenetics.
C.P. Doherty, Royal College of Surgeons in Ireland, Dublin: The potential for endophenotypic description in epilepsy syndromes.
A. Escayg, Emory University School of Medicine, Atlanta, Georgia: Role of the voltage-gated sodium channels in inherited epilepsy.

SESSION 5: Epilepsy Pharmacogenetics I
Chairperson: S.M. Sisodiya, University College London, United Kingdom

D.B. Goldstein, Duke Institute for Genome Sciences and Policy, Durham, North Carolina: Epilepsy pharmacogenetics: Where are we headed?
D.L. Kroetz, University of California, San Francisco: Expression of multidrug resistance transporters in human brain and relationship to MDR polymorphisms.
D. Weinshenker, Emory School of Medicine, Atlanta, Georgia: Noradrenergic control of seizure susceptibility and anticonvulsant drug efficacy.
C. Szoeke, The University of Melbourne, Australia: Pharmacogenetics in anti-epilepsy medications: An Australian prospective study in newly treated patients.

SESSION 6: Epilepsy Pharmacogenetics II
Chairperson: D.B. Goldstein, Duke Institute for Genome Sciences and Policy, Durham, North Carolina

M. Pirmohamed, University of Liverpool, United Kingdom: Epilepsy pharmacogenetics.
R.A. Radtke, Duke University Medical Center, Durham, North Carolina: Defining treatment response in epilepsy pharmacogenetics.
A.C. Need, Duke Institute for Genome Sciences and Policy, Durham, North Carolina: Pharmacogenetics of topiramate-induced cognitive deficits in epilepsy patients.
A. Malhotra, The Zucker Hillside Hospital, Glen Oaks, New York: Genes for neurocognitive function: Implications for treatment.

The GABAergic System

October 30–November 2

FUNDED BY **Marie Robertson Memorial Fund**

ARRANGED BY **Z.J. Huang**, Cold Spring Harbor Laboratory
G. Buzsáki, Rutgers, The State University of New Jersey

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Z.J. Huang, Cold Spring Harbor Laboratory

SESSION 1: Cell Types: From Genomics (Genotypes) to Phenotypes
Chairperson: B. Connors, Brown University, Providence, Rhode Island

H. Markram, Brain & Mind Institute, Lausanne, Switzerland: Reconstructing the neocortical microcircuit with a diversity of GABAergic interneurons.

S. Nelson, Brandeis University, Waltham, Massachusetts: Physiological genomics of cortical interneurons

SESSION 2: Development
Chairperson: D.A. McCormick, Yale University School of Medicine, New Haven, Connecticut

G.J. Fishell, Skirball Institute, New York: The developmental origin of cortical interneurons predicts their mature physiological properties.

S.A. Anderson, Weill Medical College of Cornell University, New York: Specification of cortical interneurons in the medial ganglionic eminence.

A. Represa, INMED/INSERM, Marseille, France: GABA is the pioneering transmitter in developing hippocampus.

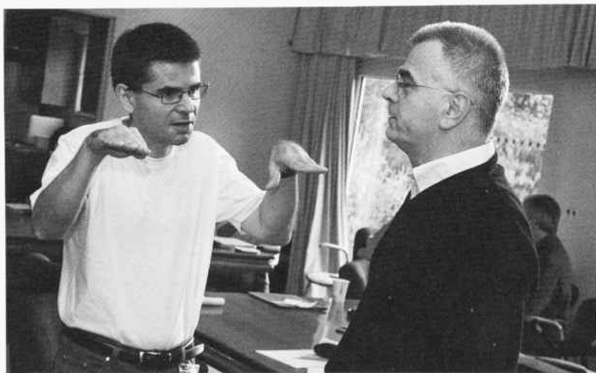
SESSION 3: GABA Synthesis and Release, Transport
Chairperson: I. Mody, University of California, Los Angeles

Z.J. Huang, Cold Spring Harbor Laboratory: A novel function of GABA in regulating GABAergic synapse and circuit development.

K. Behar, Yale University School of Medicine, New Haven, Connecticut: Measurements of GABA synthesis and GABA/glutamine cycling in relation to the GAD isoforms in rat cerebral cortex using magnetic resonance spectroscopy.

Y. Zilberter, Institute de Neurobiologie de la Mediterranee, Marseille, France: Pyramidal cell and interneurons in layer 2/3 of the juvenile neocortex: Multifaceted relations.

D. Attwell, University College London, United Kingdom: Reversal of GABA transporters and plasticity of GABA receptor signaling in brain ischemia.



P. Jonas, G. Buzsaki

SESSION 4: GABA Receptors I
Chairperson: D. Attwell, University College London, United Kingdom

R.W. Olsen, University of California, Los Angeles: GABA-A receptors: Identification of the alcohol receptor as an extrasynaptic subtype of GABA-A receptors.
U. Rudolph, University of Zurich, Switzerland: GABA-A receptor subtypes: Targets for novel anxiolytic and antipsychotic drugs.
I. Mody, University of California, Los Angeles: Distinct modulation of tonic and phasic inhibitions.

SESSION 5: GABA Receptors II
Chairperson: D.M. Kullman, University College London, United Kingdom

S. Moss, University of Pennsylvania, Philadelphia: Molecular mechanisms that regulate the membrane trafficking and function of GABA receptors.
B. Bettler, Universital Basel, Switzerland: Genetic dissociation of GABA-B functions.
W. Sieghart, Center for Brain Research, Vienna, Austria: Defining the role of neuronal circuits: Fast and reversible regulation of selected cell types.

SESSION 6: Cellular Physiology 1
Chairperson: M. Hausser, University College London, United Kingdom

D.A. McCormick, Yale University School of Medicine, New Haven, Connecticut: GABAergic systems critically control spike rate and timing in cortical networks.
B.W. Connors, Brown University, Providence, Rhode Island: Coordinating GABAergic networks with chemical and electrical synapses.
A.M. Thomson, University of London, United Kingdom: Interneurons and inhibitory circuitry in cortical regions.

SESSION 7: Cellular Physiology II
Chairperson: S. Andersen, Weill Medical College of Cornell University, New York

M. Hausser, University College London, United Kingdom: Functional properties of inhibitory circuits in the cerebellar cortex.
E.M. Callaway, The Salk Institute for Biological Studies, La Jolla: Fine-scale and inhibitory cell-type specificity of cortical connections.
P. Jonas, Universitat Freiburg, Germany: Fast, strong, and shunting inhibitory synapses improve the robustness of γ oscillations in hippocampal interneuron networks.

SESSION 8: Plasticity
Chairperson: A. Graybiel, Massachusetts Institute of Technology, Cambridge

M.-M. Poo, University of California, San Diego: Plasticity of GABAergic transmission.
D.M. Kullmann, University College of London, United Kingdom: Plasticity of GABAergic inhibition of hippocampal interneurons.
C. McBain, NICHD, Bethesda, Maryland: Plasticity at hippocampal mossy fiber interneuron synapses: What goes down sometimes must go back up!

SESSION 9: Network, System, Behavior - Part 1
Chairperson: M.-M. Poo, University of California, San Diego

G. Buzsáki, Rutgers, The State University of New Jersey, Newark: Functions and cost of inhibition: Questions for the future.
H. Monyer, University of Heidelberg, Germany: Molecular approaches to study GABAergic interneurons at the cellular and systems level.
X. Wang, Brandeis University, Waltham, Massachusetts: Interneuron actions in a working memory network: To inhibit or disinhibit?

SESSION 10: Network, System, Behavior: Part 2 (Striatum, Basal Ganglia, and "Other Systems")
Chairperson: K. Behar, Yale University School of Medicine, New Haven, Connecticut

B. Rudy, New York University: Molecular determinants of fast-spiking cell function.
A. Graybiel, Massachusetts Institute of Technology, Cambridge: Inhibition in the basal ganglia.
D.A. Lewis, University of Pittsburgh, Pennsylvania: Cortical GABA neurons and the pathophysiology of schizophrenia.
G. Buzsáki, Rutgers, The State University of New Jersey, Newark: Summary.

Barriers and Solutions in the Use of Mouse Models to Develop Therapeutic Strategies for NF1- and NF2-associated Tumors

November 3–5

FUNDED BY **U.S. Department of Defense (through a grant to the Children's Tumor Foundation)**

ARRANGED BY **K.M. Shannon**, University of California
K. Hunter-Schaedle, Children's Tumor Foundation

SESSION 1: Setting the Stage
Chairperson: K.M. Shannon, University of California

K.M. Shannon, University of California, San Francisco: Overview of meeting purpose and goals: "Throwing down the gauntlet."

M. Kaime, Congressionally Directed Medical Research Program, Fort Detrick, Maryland: Plans for the formation of a Neurofibromatosis Clinical Research Consortium.

S. Lowe, Cold Spring Harbor Laboratory: The use of mouse models to probe drug sensitivity and resistance.

D.A. Tuveson, University of Pennsylvania School of Medicine, Philadelphia: A prototype mouse hospital for performing preclinical trials.

Discussion moderated by K.M. Shannon, University of California, San Francisco

N. Ratner, A. Bernards

SESSION 2: NF1- and NF2-associated Tumors: Clinical and Pathologic Features and Current Treatments
Chairperson: D.W. Clapp, Indiana University School of Medicine, Indianapolis

M. MacCollin, Massachusetts General Hospital, Charlestown: Clinical aspects of neurofibromatosis demanding attention.
B. Welling, The Ohio State University, Columbus: Barriers and solutions in the use of mouse models to develop strategies for NS1- and NS2-associated tumors.
S. Blaney, Baylor College of Medicine, Houston, Texas: Challenges in clinical trial development for childhood cancers and applicability to neurofibromatosis-related tumors.
A. Stemmer-Rachamimov, Massachusetts General Hospital, Boston: Of mice and men: Pathology of neurofibromatosis-associated lesions.
B. Widemann, National Cancer Institute, Bethesda, Maryland: Endpoints for clinical trials in NF.

Discussion moderated by W. Clapp, Indiana University School of Medicine, Indianapolis

SESSION 3: Therapeutic Targets and Drug Discovery in NF1: Part 1
Chairperson: M. MacCollin, Massachusetts General Hospital, Charlestown

A. Bernards, Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown: NF1 drug targets from a fly's perspective.
N. Ratner, Children's Hospital Medical Center, Cincinnati, Ohio: Transgenic and cell culture models of NF1.

SESSION 4: Target and Drug Discovery in NF1: Part 2
Chairperson: B. Widemann, National Cancer Institute, Bethesda, Maryland

K. Cichowski, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts: mTOR as a potential therapeutic target in NF1.
J. Gibbs, Merck & Co., Inc., Boston, Massachusetts: Thinking beyond Ras GTPase.
G. Bollag, Plexxikon, Berkeley, California: Kinase inhibitors for the potential treatment of NF1.

Discussion moderated by M. MacCollin, Massachusetts General Hospital, Charlestown, and **B. Widemann,** National Cancer Institute, Bethesda, Maryland

SESSION 5: Therapeutic Targets and Drug Discovery in NF2
Chairperson: B. Welling, The Ohio State University, Columbus

J. Chernoff, Fox Chase Cancer Center, Philadelphia, Pennsylvania: A search for allosteric inhibitors of p21-activated kinases.
A. McClatchey, Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown: Preclinical therapeutics for NF2: Are we ready?
R. Chen, NexGenix Pharmaceuticals, LLC, Burlingame, California: Small-molecule inhibitors of Pak for the treatment of NF2.

Discussion moderated by B. Welling, The Ohio State University, Columbus

SESSION 6: Mouse Models and Preclinical Data
Chairperson: B.R. Korf, University of Alabama, Birmingham

E. Holland, Memorial Sloan-Kettering Cancer Center, New York: Gliomas.
D. Gutmann, Washington University School of Medicine, St. Louis, Missouri: Mouse models of NF-1-associated optic glioma.
D.W. Clapp, Indiana University School of Medicine, Indianapolis: The use of PET/CT imaging to detect the development of plexiform neurofibromas in Krox20; Nf1flox/-mice.
K. Shannon, University of California, San Francisco: Stage-specific response of NF1 mutant myeloid malignancies to a targeted agent.
M. Giovannini, "Génomique Fonctionnelle des Tumeurs Solides," Paris, France: Understanding F2: Insights from mouse models.

Discussion moderated by B.R. Korf, University of Alabama, Birmingham

K. Hunter-Schaedle, Children's Tumor Foundation, New York: Children's Tumor Foundation Drug Discovery Partnerships: A proposed partnering and funding initiative.

SESSION 7: Breakout Sessions

Introduction by Moderators: K.M. Shannon, University of California, San Francisco, and **D.H. Gutmann,** Washington, University School of Medicine, St. Louis, Missouri

Each participant was assigned by the organizers to a breakout session as follows:

Group 1: What additional information do we need to have regarding drug targets in NF1 and NF2? How can we use tissues from genetically engineered mice to address this problem?

Assigned Moderator: A. Bernards, Massachusetts General Hospital Cancer Center, Charlestown

J. Chernoff, Fox Chase Cancer Center, Philadelphia, Pennsylvania
K. Chichowski, Brigham & Women's Hospital, Boston, Massachusetts
A. McClatchey, Massachusetts General Hospital Cancer Center, Charlestown
N. Ratner, Children's Hospital Medical Center, Cincinnati, Ohio

Group 2: What are the major barriers to conducting therapeutic trials in NF patients?

Assigned Moderator: G. Bollage, Plexxicon, Berkeley, California

M. MacCollin, Massachusetts General Hospital, Charlestown
A. Stemmer-Rachamimov, Massachusetts General Hospital, Charlestown
P. Bellermann, Children's Tumor Foundation, Sherman, Texas

Group 3: What questions can we address with mouse models that we cannot address by studying human tumors?

Assigned Moderator: M. Giovannini, "Génomique Fonctionnelle des Tumeurs Solides," Paris, France

D.H. Gutmann, Washington University School of Medicine, St. Louis, Missouri
M. McLaughlin, Massachusetts Institute of Technology Center for Cancer Research, Cambridge
D.W. Clapp, Indiana University School of Medicine, Indianapolis

Group 4: How can we maximize interactions with industry to identify and evaluate new therapies for NF?

Assigned Moderator: J.B. Gibbs, Merck & Co., Inc., Boston, Massachusetts

R.-H. Chen, NexGenix Pharmaceuticals LLC, Burlingame, California
E. Holland, Memorial Sloan-Kettering Cancer Center, New York
C. Marks, National Cancer Institute, Bethesda, Maryland
K. Hunter-Schaedle, Children's Tumor Foundation, New York

Group 5: How can we design preclinical trials to provide useful information for human clinical trials?

Assigned Moderator: S. Blaney, Baylor College of Medicine, Houston, Texas

K.M. Shannon, University of California, San Francisco
D.A. Tuveson, University of Pennsylvania School of Medicine, Philadelphia
B. Widemann, National Cancer Institute, Bethesda, Maryland
J. Heemskerk, NINDS, NIH, Bethesda, Maryland

SESSION 8: Final Recommendations

Open Discussion and Final Recommendations

Moderators: K.M. Shannon, University of California, San Francisco, and **D.H. Gutmann,** Washington University School of Medicine, St. Louis, Missouri

A. Bernards, Massachusetts General Hospital Cancer Center, Charlestown: Group 1 report.
D. Ingram, Indiana University School of Medicine, Indianapolis: Group 2 report.
M. Giovannini, "Génomique Fonctionnelle des Tumeurs Solides," Paris, France: Group 3 report,
J.B. Gibbs, Merck & Co., Inc., Boston, Massachusetts: Group 4 report.
S. Blaney, Baylor College of Medicine, Houston, Texas: Group 5 report.

The Biology and Practice of Mammalian Cloning: A Reassessment

November 8–11

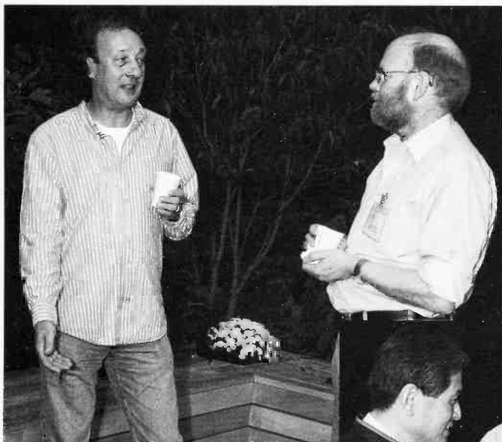
FUNDED BY **Richard Lounsbery Foundation, Inc.**

ARRANGED BY **P. Mombaerts**, The Rockefeller University
I. Wilmut, University of Edinburgh

Welcome and Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Introduction

S. Willadsen, Saint Barnabas Medical Center, Livingston, New Jersey: Cloning and interspecific chimeras.
I. Wilmut, University of Edinburgh, United Kingdom: Cells from cloned embryos: Implications of observations in livestock.
A. Trounson, Monash Immunology and Stem Cell Laboratories, Clayton, Victoria, Australia: Cloning by modification of donor cells and cell fusion techniques.
M.E. Westhusin, Texas A&M University, College Station: An update on cloning at Texas A&M: White-tailed deer and transgenic goats.



K. Campbell, I. Wilmut

W.-S. Hwang, G. Schatten, X. Yang

SESSION 2: Human Nuclear Transfer and Embryonic Stem Cells

W.-S. Hwang, Seoul National University, Korea: Human nuclear transfer I: Background and status.

S.K. Kang, Seoul National University, Korea: Human nuclear transfer II: Technical advances.

G.E. Schatten, University of Pittsburgh, Pennsylvania: Scientific frontiers enabled by patient-specific, disease-specific, and primate-specific stem cells established using nuclear transfer: How to accelerate stem cell biomedical breakthroughs globally.

L. Studer, Memorial Sloan-Kettering Cancer Center, New York: Human embryonic stem cells and therapeutic cloning.

A.H. Brivanlou, The Rockefeller University, New York: In vivo assay of human ES cells.

SESSION 3: Mechanisms of Reprogramming

M. Boiani, Max-Planck Institut für Molekulare Biomedizin, Munich, Germany: Is it all about epigenetics? A look at nuclear organization, chromosome transmission, and genetic mutation in clonal mouse embryos.

A. Bortvin, Carnegie Institution of Washington, Baltimore, Maryland: Development of the next generation of experimental approaches to the epigenetic regulation of the genome.

K. Campbell, University of Nottingham, United Kingdom: Oocyte kinase and development in ovine nuclear transfer embryos.

P. Collas, University of Oslo, Norway: In vitro manipulation of donor nuclei and cells prior to cloning.

SESSION 4: Mouse and Rat

P. Mombaerts, The Rockefeller University, New York: Olfaction targeted.

K. Eggan, Harvard University, Cambridge, Massachusetts: Cloning and stem cells: Interrogating development and disease by nuclear transplantation.

K. Inoue, RIKEN, Tsukuba, Ibaraki, Japan: Cloning mice from differentiated and undifferentiated cells.

T. Yagi, Osaka University, Japan: Mouse cloning with neuronal nuclei.

P.M. Iannaccone, Northwestern University Medical School, Chicago, Illinois: The isolation and use of rat ES as potential nuclear donor cells in NT cloning of the rat.

SESSION 5: Other Species, Ethics

J. Cibelli, Michigan State University, East Lansing: Gene expression of reprogrammed bovine NT embryos.

C. Galli, Universita Di Bologna, Cremona, Italy: Reprogramming somatic cells for embryonic, fetal, and offspring development in large animals.

X. Yang, University of Connecticut, Storrs: The health status of our cloned cattle as well as their offspring and their organ/product compositions.

Y. Hosoi, Kinki University, Naga, Wakayama, Japan: In vitro development of macaca-rabbit-cloned embryos and trials of establishment of their cell lines.

D. Spar, Harvard Business School, Boston, Massachusetts: The business of stem cells.

The Intracellular Molecular Environment

November 13–16

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **D. Spector**, Cold Spring Harbor Laboratory
J. Swedlow, University of Dundee

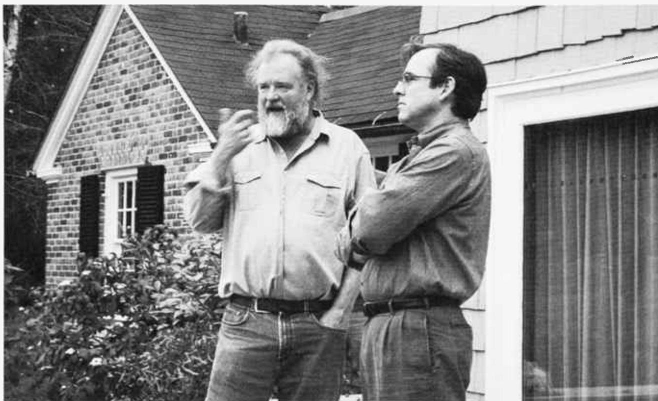
Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Nuclear Dynamics and Organization
Chairperson: T. Pederson, University of Massachusetts Medical School, Worcester

J. Gall, Carnegie Institution of Washington, Baltimore, Maryland: Some physical properties of the nucleoplasm and nuclear organelles.
T. Cremer, Ludwig-Maximilians-Universität München, Germany: Mapping nuclear architecture in space and time.
A. Belmont, University of Illinois, Urbana-Champaign: Large-scale chromatin structure and dynamics.
D. Spector, Cold Spring Harbor Laboratory: Nuclear dynamics.
T. Pederson, University of Massachusetts Medical School, Worcester: Matriculation of mRNA in the nucleus: The movements are in the medium.
R. Hancock, Laval University, Canada: The crowded nucleus.

SESSION 2: Interpreting Intracellular Movement
Chairperson: K. Luby-Phelps, University of Texas Southwestern Medical Center, Dallas

K. Luby-Phelps, University of Texas Southwestern Medical Center, Dallas: Physical constraints on the biochemistry of the cell interior.
M. Saxton, University of California, Davis: A biological interpretation of anomalous subdiffusion.
S. Schnell, Indiana University, Bloomington: Deterministic and stochastic kinetics of reactions occurring in crowded intracellular environments.
M. Weiss, Deutsches Krebsforschungszentrum, Heidelberg, Germany: Anomalous diffusion caused by molecular crowding.
A. Elcock, University of Iowa, Iowa City: Molecular simulations of diffusion and association under pseudocellular conditions.
G. Odell, University of Washington, Seattle: Mathematical/computer modeling as a tool for comprehending cytoskeletal dynamics.



G. Odell, P. Sorger

SESSION 3: Cytoplasmic Dynamics and Signaling
Chairperson: P. Sorger, Massachusetts Institute of Technology, Cambridge

J. Marko, University of Illinois, Chicago: Mechanics of DNA-protein complexes and whole chromosomes.
M. Engstler, Ludwig-Maximilians-Universität, München, Germany: Navigation within a tiny but tidy cell: What can we learn from trypanosomes?
C. Weijer, University of Dundee, United Kingdom: Signaling to the cytoskeleton during chemotactic cell movement.
S. Altschuler, University of Texas Southwestern Medical Center, Dallas: Creating and maintaining asymmetries.
P. Sorger, Massachusetts Institute of Technology, Cambridge: Modeling cell signaling circuits.

SESSION 4: Modeling Dynamic Movements
Chairperson: G. Danuser, Scripps Research Institute, La Jolla, California

D. Eggers, San José State University, California: Amplified hydration effects on binding equilibria in vivo.
E. Siggia, The Rockefeller University, New York: Fluctuations in the cell cycle in yeast.
K. Schulten, University of Illinois, Urbana-Champaign: In situ molecular modeling of cellular processes.
G. Danuser, Scripps Research Institute, La Jolla, California: The dynamic regulation of two colocalized, yet functionally distinct actin arrays in cell migration.
R. Singer, Albert Einstein College of Medicine, Bronx, New York: Diffusion vs. transport: Where and when?

SESSION 5: Photons, Electrons, and Protons: Visualizing Intracellular Dynamics
Chairperson: M. Ellisman, University of California, San Diego

G. Dunn, King's College, London, United Kingdom: Using FLAP to find out how molecules get to where they are needed.
M. Ellisman, University of California, San Diego: Toward the visible cell: Collecting and connecting mesoscale data.
G. Pielak, University of North Carolina, Chapel Hill: In-cell NMR.
J. Swedlow, University of Dundee, United Kingdom: Mechanistic studies of nuclear and chromosome dynamics.

The Biology of Neuroendocrine Tumors

November 20–22

FUNDED BY **Verto Institute**

ARRANGED BY **A.J. Levine**, Institute for Advanced Study
E. Vosburgh, Verto Institute

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Biology
Chairperson: A.J. Levine, Institute for Advanced Study, Princeton, New Jersey

S.K. Kim, Stanford University School of Medicine, California: Mechanism of islet tumor suppression by MEN-1.
O. Rosen, Dana Farber Cancer Institute, Boston, Massachusetts: Chromatin-modifying and RNA processing complexes of the parafibromin neuroendocrine tumor suppressor protein.
X. Hua, University of Pennsylvania, Philadelphia: Coordinated regulation of cell proliferation and apoptosis in endocrine cells by the tumor suppressor menin.
M. Yen, Stanford University Medical Center, California: Hormonal regulation of menin: Opportunities for therapy?
C.J. Barnstable, Yale University School of Medicine, New Haven, Connecticut: Therapeutic potential of PEDF in controlling the growth of neural tumors.
J. Tobram-Tink, University of Missouri, Kansas City: PEDF blocks VEGFR2 signaling and angiogenesis: Potential for reducing tumor growth and metastasis.

R. Sackler, S. Kaufer

Keynote Speaker: J. Mendelsohn, University of Texas M.D. Anderson Cancer Center, Houston: EGF receptors: A target for cancer therapy.

SESSION 2: Genetics
Chairperson: E. Vosbugh, Verto Institute, Stamford, Connecticut

A.J. Levine, Institute for Advanced Study, Princeton, New Jersey: IAS/CINJ: SNPs in the *p53* pathway.
A. Rashid, University of Texas M.D. Anderson Cancer Center, Houston: Genome-wide SNP allelotyping in carcinoid tumors and pancreatic endocrine tumors.
E. Freed, Dana-Farber Cancer Institute, Boston, Massachusetts: Use of SNPs to identify genes in neuroendocrine tumor growth and development.
M. Essand, Uppsala University, Sweden: Gene therapy and immunotherapy of gastrointestinal neuroendocrine tumors.
F. Leu, Verto Institute, Princeton, New Jersey: CINJ: SSTR1-5 antibodies.
C. Harris, Verto Institute, Princeton, New Jersey: CINJ: LINE 1 retrotransposons and genomic stability, role in neuroendocrine tumors?
C. Kuperwasser, Tufts University, Boston, Massachusetts: Novel xenograft models of human breast cancer.

SESSION 3: Progress in the Treatment of Carcinoid Tumors
Chairperson: L.K. Kvols, University of South Florida, Tampa

D. Hochhauser, Royal Free & University College Medical School, London, United Kingdom: Modulation of chemotherapy by EGFR inhibition.
R.V. Lloyd, Mayo Clinic, Rochester, Minnesota: EGFR studies in GI carcinoids and pancreatic tumors and response to therapy.
L.K. Kvols, University of South Florida, Tampa: Clinical updates in carcinoid therapy.
J. Yao, Gastrointestinal Medical Oncology, Houston, Texas: Translational research at M.D. Anderson.
M.H. Kulke, Dana-Farber Cancer Institute, Boston, Massachusetts: Results of the phase II trial of SU11248 in metastatic neuroendocrine tumors.

Prion Biology: Puzzles and Paradoxes

November 27–30

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Collinge,** University College
C. Weissmann, The Scripps Institute

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: The Species Barrier and Prion Strains
Chairperson: J. Collinge, National Hospital for Neurology & Neurosurgery, London, United Kingdom

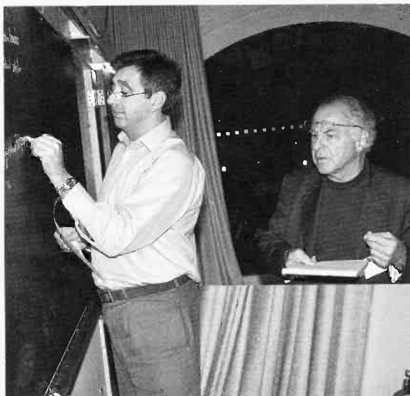
K. Wuthrich, The Scripps Research Institute, La Jolla, California: Structural biology of the cellular form of prion proteins.

J. Wadsworth, MRC Prion Unit, University College, London, United Kingdom: Molecular basis of species- and strain-dependent barriers in transmissibility of mammalian prions.

J. Manson, Institute for Animal Health, Edinburgh, United Kingdom: Control of TSEs by host PrP.

M.H. Groschup, Friedrich-Loeffler Institute, Greifswald-Insel Riems, Germany: Atypical scrapie cases in small ruminants carry abnormal PrP with unusual biochemical features.

W.K. Surewicz, Case Western Reserve University, Cleveland, Ohio: Molecular basis of species and strain-dependent barriers in transmissibility of mammalian prions.



J. Collinge, C. Weissmann

K. Wuthrich, H. Michel

S. Prusiner, C. Beisel

SESSION 2: How Did Prions Evolve and Epidemics Originate?
Chairperson: C. Weissmann, The Scripps Institute, Jupiter, Florida

S. Mead, MRC Prion Unit, University College, London, United Kingdom: The genetic consequences of prion disease epidemics.

R.B. Wickner, National Institutes of Health, Bethesda, Maryland: Transformation of (URE3) by amyloid from recombinant Ure2p transmits three prion variants.

S.L. Lindquist, Whitehead Institute, Cambridge, Massachusetts: Structural insights into yeast prion conversion.

L.B. Schonberger, DVRD, NCID, CDC, Atlanta, Georgia: Surveillance of Creutzfeldt-Jakob disease in the United States.

SESSION 3: Key Issues Arising from the Day

SESSION 4: Synthetic Prions: Have We Made Them?
Chairperson: C. Weissmann, The Scripps Institute, Jupiter, Florida

S.B. Prusiner, University of California, San Francisco: Mouse synthetic prions I.

G. Legname, University of California, San Francisco: Mouse synthetic prions II.

I. Baskakov, University of Maryland Biotechnology Institute, Baltimore: Mechanisms of PrP polymerization into amyloid fibrills.

G. Jackson, National Hospital for Neurology & Neurosurgery, London, United Kingdom: Assaying for synthetic prions.

G. Telling, University of Kentucky, Lexington: Transgenic studies of CWD and mechanisms controlling prion transmission.

B. Caughey, NIAID, National Institutes of Health, Hamilton, Montana: Particle size and infectivity in TSE diseases.

S. Supattapone, Dartmouth Medical School, Hanover, New Hampshire: PrPres formation from purified substrates in vitro.

C. Soto, University of Texas Medical Branch, Galveston: Generation of prions, species barrier, and prion strains.

SESSION 5: Key Issues Arising From the Day

SESSION 6: Neurotoxicity and Therapeutics in Neurodegenerative Disease
Chairperson: J. Collinge, MRC Prion Unit, University College, London, United Kingdom

C.I. Lasmezas, The Scripps Institute, Jupiter, Florida: Mechanisms of prion-induced neurodegeneration.

D. Caspar, Florida State University, Tallahassee: Prion amyloid fibrils and cross-β confusion.

D.B. Teplow, David Geffen School of Medicine at UCLA: Amyloid B-protein asembly and neurodegeneration: When the core of the problem is not the "core."

SESSION 7: Key Issues for Future Research

Cancer Stem Cells

December 4–7

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M. Wicha**, University of Michigan
J. M. Rosen, Baylor College of Medicine

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Key Questions To Be Addressed: J.M. Rosen, Baylor College of Medicine, Houston, Texas

SESSION 1: Key Signaling Pathways and Mechanisms in Stem Cell Self-renewal
Chairperson: T.D. Tlsty, University of California, San Francisco

R. Nusse, Stanford University Medical School, California: *Wnt* signaling and stem cell control.

P. Beachy, Johns Hopkins University, Baltimore, Maryland: *Hedgehog* signaling in tissue repair, neoplasia, and metastasis.

P. Polakis, Genentech, Inc., San Francisco: Therapeutic intervention of *Wnt* signaling in cancer.

J. Sherley, Massachusetts Institute of Technology, Cambridge: Adult stem cells as sites of carcinogenesis.

I.R. Lemischka, Princeton University, New Jersey: Functional genomics and stem cells.

M. van Lohuizen, The Netherlands Cancer Institute, Amsterdam: Polycomb repressors controlling stem cell fate: Implications for cancer and development.

SESSION 2: Role of the Microenvironment and the Stem Cell Niche
Chairperson: R.J. Jones, Johns Hopkins University, Baltimore, Maryland

G.H. Smith, NCI/National Institutes of Health, Bethesda, Maryland: The influence of the mammary stem cell niche on borrowed stem cells.

T.D. Tlsty, University of California, San Francisco: Early epigenetic and genetic events in carcinogenesis.

M. Wicha, J. Chang

SESSION 3: Hematopoietic Stem Cells, Leukemias, and Myeloma
Chairperson: M. van Lohuizen, The Netherlands Cancer Institute, Amsterdam

M. Goodell, Baylor College of Medicine, Houston, Texas: Regulation of hematopoietic stem cell self-renewal.
J.E. Dick, Princess Margaret Hospital, Toronto, Canada: Cancer stem cells: Lessons from leukemia.
S.J. Morrison, University of Michigan Medical School, Ann Arbor: Role of *Pten* in the self-renewal of normal and leukemic stem cells.
G. Morrone, University of Catanzaro, Italy: Dissecting leukemogenesis: Primary leukemic stem cells and gene-transfer-mediated models of leukemogenesis.
T. Look, Dana Farber Cancer Institute, Boston, Massachusetts: del(5q) and epigenetic suppression of α-caterin (CTNNA1) in CD34+CD38 human AML-initiating cells.
C. Jordan, University of Rochester School of Medicine, New York: Novel strategies for selective eradication of leukemia stem cells.
R.J. Jones, Johns Hopkins University, Baltimore, Maryland: Cancer stem cells: Clinical implications.

SESSION 4: Solid Cancers 1
Chairperson: J.M. Rosen, Baylor College of Medicine, Houston, Texas

O.N. Witte, University of California, Los Angeles: Prostate stem cells and prostate cancer.
J.M. Bishop, University of California, San Francisco: Progenitor cells and tumorigenesis in the liver and breast: A study with mouse models.
P. Dirks, Hospital for Sick Children, Toronto, Canada: Human brain-tumor-initiating cells.

SESSION 5: Clinical Implications
Chairperson: J.M. Rosen, Baylor College of Medicine, Houston, Texas

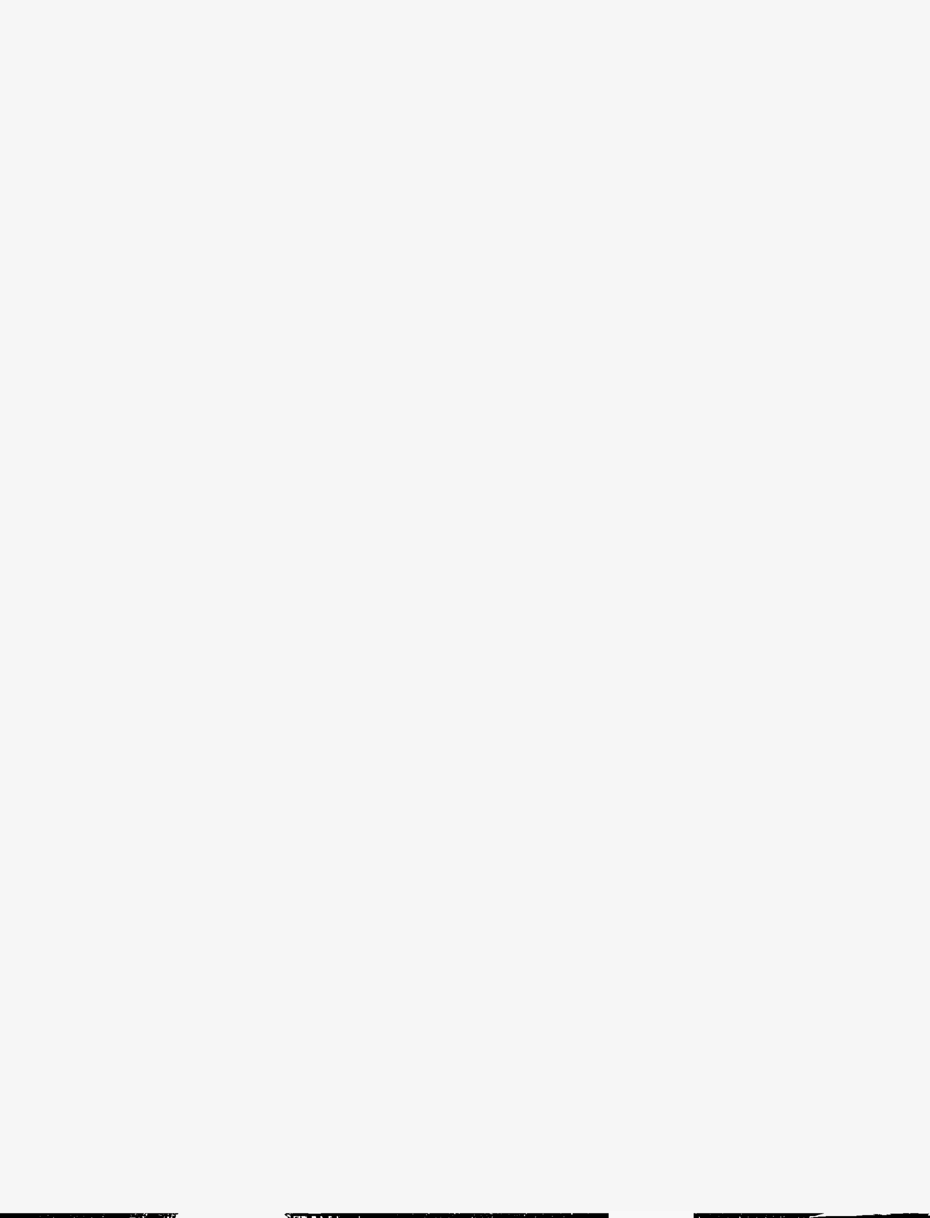
L. Norton, Memorial Sloan-Kettering Cancer Center, New York: Is cancer a disease of self-seeding (by cancer stem cells?).
J. Chang, Baylor College of Medicine, Houston, Texas: Breast cancer stem cells and therapeutic resistance.
G.V. Glinsky, Ordway Research Institute, Inc., Albany, New York: A death from cancer pathway and stem cell cancer hypothesis.

SESSION 6: Solid Cancers 2
Chairperson: M. Wicha, University of Michigan, Ann Arbor

C. Alexander, University of Wisconsin, Madison: Mouse mammary development and neoplasia.
M.T. Lewis, Baylor College of Medicine, Houston, Texas: Hedgehog regulation of mammary epithelial stem/progenitor cells in the mouse.
M.F. Clarke, University of Michigan Health System, Ann Arbor: Identification and molecular characterization of epithelial cancer stem cells in human and mouse solid tumors.
F.M. Watt, Cancer Research, United Kingdom, London: Role of stem cells and differentiated cells in epidermal carcinogenesis.
M. Wicha, University of Michigan, Ann Arbor: Role of Hedgehog and Bmi-1 signaling in mammary stem cell self-renewal.
R. Clarke, Christie Hospital Trust, Manchester, United Kingdom: Isolation and characterization of human breast stem cells.
A.A. Dlugosz, University of Michigan, Ann Arbor: Hedgehog signaling in tumor initiation and maintenance.
R. McKay, NINDS/National Institutes of Health, Bethesda, Maryland: Stem cell survival mechanisms in regeneration and cancer.

SESSION 7: Key Issues of the Meeting
Moderators: M. Wicha, University of Michigan, Ann Arbor and **J.M. Rosen,** Baylor College of Medicine, Houston, Texas

DOLAN DNA LEARNING CENTER



DOLAN DNA LEARNING CENTER EXECUTIVE DIRECTOR'S REPORT

Preparing students and families to thrive in the gene age

ADMINISTRATION	INSTRUCTION	BIOMEDIA	TECHNOLOGY DEVELOPMENT
Nancy Daidola	Elna Carrasco	John Connolly	Adrian Arva
Mary Lamont	Jeanette Collette	Eun-Sook Jeong	Greg Chin
Stacy Leotta	David Gundaker	Susan Lauter	Uwe Hilgert
David Micklos	Natalia Hanson	Chun-hua Yang	Bruce Nash
Karen Orzel	Laura Johns		
Carolyn Reid	Amanda McBrien		
	Erin McKechnie		
	Danielle Sixsmith		
	Lauren Weidler		

People in the region of the upper Yukon refer to their part of Alaska as "the country." A stranger appearing among them is said to have "come into the country."

In his book *Coming into the Country*, John McPhee describes his experiences in Alaska, in 1976, as people anticipated the opening of oilfields and pipelines in that last great American frontier. McPhee's Alaska of 1976 presents an appropriate metaphor for biology in 2006. Like Alaska, the landscapes of genomes laid bare present vistas of limitless promise. Mining living genomes presents many of the same logistical and philosophical problems as mining the Alaskan wilderness. Frontiers of all kinds challenge similar sorts of people—those anxious for knowledge, fortune, adventure, solitude, or another chance. The desires of those who would exploit the frontier need to be balanced against those who see it as a holy place for contemplation.

Coming into the gene age also challenges those who teach. DNA sequences from hundreds of organisms are available to anyone 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 the promise of making modern biology an egalitarian pursuit. For the first time in the history of biology, students can work with the same information, at the same time, and with the same tools as research scientists.

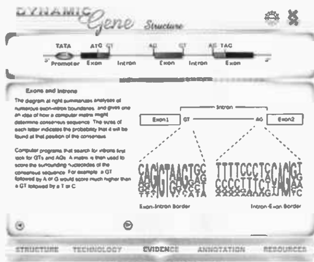
A fictitious cover design based on John McPhee's *Coming into the Country*

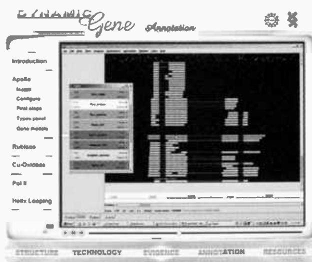
Working directly with genome data can help students understand the ever-changing concept of a gene and to conceptualize the "big picture" of a complex, dynamic genome. Evaluating genome evolution and understanding our shared genetic heritage also may be the best inoculation against racism.

Biology researchers and educators need to seize this unique opportunity to involve students in the ever-increasing trove of DNA data that will change forever how we think about life. First, we need to allow students to look at their own DNA and use it as an entrée to the genome world. Second, we need to integrate bioinformatics with biochemistry labs, so that students become adept in moving between the in vitro and in silico worlds. Third, we need to develop more intuitive, visually pleasing computer tools that engage students and allow them to quickly learn the rudiments of gene analysis. Fourth, we need to welcome students as partners in the effort to evaluate and annotate the vast number of genes that are known only as predicted computer models.

Coming into the Gene Age

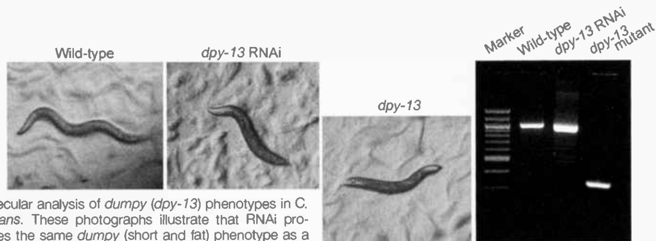
During the past year, the Dolan DNALC made significant strides to help students and teachers come into the gene age. Working directly with CSHL researchers Lincoln Stein and Doreen Ware, we completed major elements of the *Dynamic Gene* Internet site. As educational outreach for the National Science Foundation (NSF)-funded Gramene Comparative Genomics Project, this site helps students learn modern concepts of gene structure by participating in genome research. The site is essentially an educational interface to the Apollo genome annotator, a research tool for editing "gene models" predicted by computer algorithms. Apollo was used to annotate the *Drosophila* genome, and we are adapting it for use with grain plants. The site's name emphasizes the gene both as a dynamic structure that changes through evolutionary time and as a dynamic concept that changes with our increasing knowledge of genome organization. The design for *Dynamic Gene* recalls the "streamlining movement" that influenced design during the middle of the 20th century with ideas borrowed from aviation and automobile design.





Students begin with tutorials that illustrate components of genes, and how different kinds of evidence for gene structure are gathered and compiled into gene models. Narrated videos capture the screen movements and commentary as an expert uses the Apollo research tool to solve common problems of gene annotation. Students can then annotate sequences at several levels: (1) Beginners can construct gene models for individual genes, and then compare their models to a "correct" model provided by an expert annotator. (2) At the next level, students can tackle a number of genes assembled into an "artificial contig" and compare their models with curated results. (3) Advanced students, or whole classes, can take on the project of a contig containing a genome region that has not been hand-curated.

We also worked with the Carolina Biological Supply Company to develop a new line of *DNA Learning Center Kits* that stress the modern synthesis of molecular biology and computation. Each experiment in the series integrates in vitro experimentation with in silico bioinformatics. The experiments incorporate user-friendly features from the popular *DNA Science* lab-text, including flow charts, marginal notes, and extensive instructor information. Exclusive *Bio-i Guides* allow students to follow the recorded screen movements and mouse clicks of DNALC staff as they solve bioinformatics problems tailored to each experiment. A companion CD-ROM and Internet site provides exclusive access to the *Bio-i Guides* and additional e-learning tools that can be used in class or at home. Complete instructions for each experiment can be accessed as a unique virtual lab notebook, as well as printable PDF files. Animations on key techniques of molecular genetics and genomic biology are drawn from the DNALC's award-winning Internet site, *DNA Interactive*.



Molecular analysis of *dumpy* (*dpy-13*) phenotypes in *C. elegans*. These photographs illustrate that RNAi produces the same *dumpy* (short and fat) phenotype as a deletion in the *dpy-13* gene. The agarose gel compares PCR products from the *dpy-13* gene of a wild-type worm and two worms with the Dpy phenotype. The PCR products from wild-type and RNAi-induced *dpy-13* worms are identical, while the *dpy-13* mutant shows a shorter PCR product resulting from a chromosome deletion. This illustrates that RNAi silences gene function and produces a phenotype without altering the gene itself.

Bringing Revolutionary Technology into the Classroom

With the complete human DNA sequence "in the bag," ahead lies the task of understanding the function of each gene and how it interacts with other genes. Such functional studies typically rely on mutating a particular gene, and then looking for its physical or behavioral effects in a living organism. Obviously, such studies cannot be carried out on human beings, so biologists rely on "model organisms" to shed light on elements of human physiology and cell biology. Although the microscopic worm *Caenorhabditis elegans* might seem an unlikely stand-in, it actually shares quite a lot of cell biochemistry with humans and has become a key model system in which to study gene function.

In recent years, the usefulness of the *C. elegans* model system has been dramatically enhanced because it is particularly suited to the revolutionary technique of RNA interference (RNAi). This method allows one to "silence" virtually any gene at will, within the context of a living organism. Amazingly, all that is required is for the worm to eat or be bathed in double-stranded RNA that is complementary to the part of the gene to be silenced. *C. elegans* is inexpensive to maintain, grows quickly (going from egg to adult in several days), and populates a culture plate with thousands of offspring that can be quickly screened for RNAi-induced phenotypes. For these reasons, we have invested in developing the *RNAi/C. elegans* system to bring functional genome analysis into precollege and beginning college classes.

As 2005 drew to a close, we had working prototypes of key experiments for our NSF-funded curriculum that explores the mechanism and uses of RNAi. With technical support from CSHL researcher Greg Hannon, a pioneer in RNAi technology, and an Advisory Panel–lead high school and college faculty, we are prepared to test experiments at workshops to be held in San Francisco, Oklahoma, and New York City in summer 2006.

The workshop will include an entire set of techniques for doing RNAi in *C. elegans*—beginning with observation of mutant phenotypes (physical and behavioral) and basic worm "husbandry." Participants then learn simple methods to induce RNAi and observe specific mutant phenotypes in manipulated worms. The mechanism of RNAi is investigated in two *C. elegans* strains with identical "dumpy" traits: one induced by RNAi and one caused by a chromosomal deletion. "Single-worm" polymerase chain reaction (PCR) is used to amplify DNA from the *dumpy* locus, and the DNA from wild-type worms is compared to DNA from RNAi and chromosomal mutants. Gel electrophoresis identifies the deletion in the mutant, but not in wild-type and RNAi-treated strains, providing evidence that RNAi does not alter the genetic code. In another experiment, a mutant phenotype is "rescued" (compensated for) by RNAi.

Participants also learn the process needed to silence any gene in the *C. elegans* genome, providing the possibility for student-centered research projects. Participants learn to use online sequence data to design primers to amplify part of a target gene and clone it into a feeding vector. The recombinant vector is then transformed into *Escherichia coli*, which, in turn, are fed to *C. elegans*. In addition, faculty will have access to the DNALC's own collection of RNAi-feeding strains that can be used to screen for a variety of mutant phenotypes. The importance of *C. elegans* as a model for higher organisms is highlighted by bioinformatics exercises that show the strong relatedness of worm genes and their human homologs.

Building a Revolutionary Internet Site

In 2005, we began development of *Genes to Cognition (G2C) Online*, a modern Internet site on current research on the molecular basis of human thinking and disorders of thinking. The project is revolutionary because it is being built in parallel with a major international research program—its namesake *G2C* at the Wellcome Trust Sanger Institute—and because it will employ a nonlinear, network structure designed to provide a personalized learning experience. Our objective is to build an Internet site that functions on several "meta" levels in that it:

- Reports on and is informed by current neuroscience research.
- Embodies a network structure analogous to the gene and cell networks that underlie cognition.
- Encourages individuals to reflect on their own thinking and learning.

The project was launched with the first meeting of the *G2C* Advisory Panel on March 11 at the CSHL Banbury Center. The 15 participants, from the United States and Europe, brought expertise from a number of fields that will be important to the success of the project, including neuroscience research, genetics and ethics issues, precollege and college science education, informal science education, project evaluation, and multimedia production. At the meeting, panelists were introduced to the challenges of producing a network-based Internet site, including building the database/meta-tag system, evaluating the system, and disseminating the system.

We relied heavily upon concept mapping during the first year of the project. Originally developed by Advisory Panelist and education pioneer, Joseph Novak, concept maps provide a visual method to condense and represent large bodies of knowledge in a meaningful way. Thus, we worked with Joe to elicit expert concept maps from leaders in key knowledge domains of importance to *G2C Online*. Merging the individual expert maps will create a master map, whose intersections (nodes) will suggest key concepts for which rich multimedia and inquiry modules will be developed. As part of this effort, we worked with advisory panelists Laura Maitland, Mary Colvard, and Caren Gough to develop concept maps of *G2C* content areas that are of direct relevance to high school/college biology and psychology curricula.

Concept maps will guide the development of the logic system of the *knowledge network*—a system of meta-tags to annotate content. Each item of content for *G2C Online* is being built as a standalone, independent medium item that occupies a specific space in a larger network of items, or nodes. As more content is added, and as more visitors use the site, a network engine will dynamically build and rebuild the network in accordance to the relationships between each node in terms of who uses them, how they are used, and the topics they encompass. The network structure should facilitate meta-cognition by helping the visitor make connections between concepts.

Concept mapping has suggested a three-dimensional array (below) to guide initial content development and meta-tagging. Thus, if site visitors are interested in autism, they will be able to view the disorder through a number of lenses that represent a continuum of approaches to science and phenomenological levels. Thus, autism is seen not only as a disorder of behavior, but a disorder of the brain, of neural circuits that make up the brain, of cells that make up these circuits, of proteins that signal within these cells, and, finally, of genes that encode these proteins.

Cognitive Disorders	Approaches to Science	Anatomical-Molecular Continuum
ADHD	Neuroimaging	Behavioral/clinical
Alzheimer's	Electrophysiology	Anatomical: Brain regions
Autism	Psychological/clinical	Physiological: Neural circuits
Bipolar disorder	Family/epidemiological	Cell biological: Signal transduction
Chronic pain	Bioinformatics	Molecular: DNA and genetic regulation
Depression	Historical	
Schizophrenia	Ethical	
Stroke and injury		

Hewlett Grant Completes Funding for *G2C* in the U.S.

From its compelling premise—to encourage students to think about the biology of thought—to its adaptive network architecture, to its strong connections to elite centers of biological research, *G2C Online* has the potential to set a high standard for educational technology. However, to prove its real worth as a model for others, this innovative project demands an equally rigorous evaluation and dissemination program. Thus, we were pleased when, in October, the William and Flora Hewlett Foundation added $470,000 to existing funding of $1 million from the Dana Foundation to support the evaluation and dissemination of *G2C Online*. The project is funded under the technology priority of Hewlett's education program, which aims to "improve access to exemplary postsecondary and K–12 educational content through a variety of approaches." The Hewlett grant will support several major objectives:

- *Integrate insights from cognitive and neuroscience research into Internet site construction.* As part of this objective, in Fall 2006 we will convene a high-level workshop at the CSHL Banbury Center to draw together 30–40 Internet site developers and experts from diverse field to conceptualize features of Internet sites of the future.
- *Determine how concept maps can support Internet site construction and student learning.* This objective supports the construction of expert concept maps to guide creation of the *G2C* "knowledge network" and classroom studies to determine how concept mapping supports student meta-cognition.
- *Demonstrate how Internet education materials developed alongside current research efforts can support and extend syllabus-centered teaching with examples of science process.* This objective will offer insight into how supplementary Internet materials can fit into a school system that is increasingly driven by standardized testing—with a focus on Advanced Placement biology and psychology courses.
- *Provide insight into how different audiences interact with multimedia content and how narrative versus exploratory modes influence understanding of neuroscience concepts.* This objective supports research on how students and teachers make use of the *G2C* knowledge network.
- *Distribute* G2C Online *to biology and psychology educators.* This objective will support nationwide training workshops for 480 biology and psychology teachers, as well as presentations at teacher professional meetings.

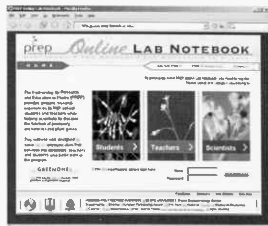
The Hewlett award puts us in the company of other high-level Internet developers. We are currently exploring partnerships with two other Hewlett grantees: public broadcaster WGBH and the Berkeley Lab's Center for Science & Engineering Education. The strength of both Dana and Hewlett support puts us in a strong position to realize our goal to obtain funding for a parallel evaluation and dissemination program in Great Britain.

Internet Collaborations Extend Our Leadership

The DNALC participated in several high-level Internet collaborations in 2005. Key among these was the announcement in October of a $2.8 million consortium grant from the National Science Digital Library program of the NSF. Under the grant, the DNALC will receive $250,000 over 4 years to catalog and make more than 100 hours of its proprietary Internet content available through the BiosciEdNet (BEN) Collaborative. Headed by the American Association for the Advancement of Science, the BEN Collaborative is primarily a cooperative of professional societies that are making their content available through the BEN portal. The DNALC is one of only several independent content producers, among more than 20 collaborators included in this second phase of the project.

The BEN Collaborative is part of the DNALC's ongoing effort to decompose its narrative content so that constituent content "molecules" and "atoms" can be searched for and viewed independently. The DNALC will make available, through the BEN Collaborative, multimedia elements from its eight major content sites. This will include approximately 200 content molecules (multimedia units, activities, lesson plans, and laboratories) and 5000 content atoms (individual multimedia items: animations, video, photographs, and flat art).

We continued to participate in the development two Internet sites funded by the National Institutes of Health (NIH). The Cancer Biomedical Informatics Grid (CaBIG, https://cabig.nci.nih.gov/) is an Internet resource to share data and tools among cancer researchers. The DNALC is lending its expertise to the Training Work Space, which is creating standards and templates to help collaborators construct a unified knowledge base. We also collaborated with the Fralin Biotechnology Center at Virginia Tech to design and program an online platform for their NIH Partnership for Research and Education in Plants (PREP; www.prep.biotech.vt.edu). This work was funded through the same NIH program that funded our *Inside Cancer* Internet site. The *PREP Online Lab Notebook* provides an environment to support student experiments with the model plant *Arabidopsis*. Students grow mutant and wild-type plants under various conditions, record their observations, and attempt to understand how mutations affect response to environmental stimuli. Plant scientists participate in the project by providing mutants and offering advice to participating students and teachers. The *PREP* Internet site coordinates with the DNALC's *Greenomes* site (www.greenomes.org), a set of molecular genetic experiments with *Arabidopsis* and corn.



Real and Virtual Visitors Increase

Real and virtual visitors continued to increase in 2005. During the year, the DNALC had 38,654 visitors, a 3% increase from the previous year. Key among these were 29,910 students and teachers who conducted lab experiments at the DNALC, in local schools, and at training workshops at sites around the world. Visits to the DNALC's family of Internet sites rose 13%, to 6.15 million. *DNA Interactive,* registered the largest increase (48%), followed by *Your Genes, Your Health* (32%) and *Genetic Origins* (28%). Much of this growth was due to a sophisticated indexing system that makes multimedia content more "visible" to search engines that are accustomed to html and text documents.

Internet Site	Average visit length (in minutes)	Visits in 2005	Increase from 2004 (%)
Gene Almanac	8:37	2,110,416	–1.20%
DNA from the Beginning	8:58	1,355,681	7.18%
Your Genes, Your Health	7:26	1,139,401	32.40%
DNA Interactive	7:27	964,919	48.43%
Image Archive on the American Eugenics Movement	8:54	286,403	1.06%
Bioservers	16:03	159,228	15.39%
Genetic Origins	7:38	140,318	28.71%
All Sites	**9:18**	**6,156,366**	**13.13%**

Taking into account that 60% of American Internet users now use high-speed connections, the DNALC updated two of its older Internet sites in Flash MX, the media integration standard for broadband. The Internet portal *Gene Almanac* was entirely reconceived. A scrolling "belt" across the middle of the screen provides access to nine DNALC Internet sites, and examples of more than 1000 multimedia items load randomly in a showcase window at top right. A drawing of the DNALC facility anchors the lower part of the screen along with access to DNALC services, and the correct phase of the moon plies the deep blue background above. *Image Archive on the American Eugenics Movement*, originally released in 2000, received a face-lift that gave it enhanced search features and added cross-referencing and interactive images. Users can now sort images by topic, time period, object type, and originating archive. Quick links allow users to access images from the same topic, time period, or archive. Image viewing has been simplified by Zoomify, which allows users to seamlessly move between low- and high-resolution images. (Rather than loading separate "normal" and "detailed" image files, Zoomify loads a single high-resolution image.) Visitors then interact with the image—panning across the image and zooming in at any point.

Students participating in camps during summer 2005

Faculty Fellowships and Training

With the support from NSF and U.S. Department of Agriculture plant research programs, we hosted five biology faculty and two student fellows in the summer of 2005. Three faculty participated in the development of the *Dynamic Gene* Internet site: Robert Wheeler of Pine Creek High School (Colorado Springs), Dr. Debra Burhans of Canisius College (Buffalo, New York), and Cesar Gutierrez of John H. Reagan High School (Austin, Texas).

The second program, "Building Leadership to Expand Participation of Underrepresented Minorities in Plant Genetics and Genomics" is the educational outreach component of genome research conducted by CSHL researcher Dick McCombie. Two pairs of faculty-student fellows who spent 3 weeks at Cold Spring Harbor were funded by this NSF grant: Dr. Mary Smith and Timothy Raines from North Carolina Agricultural and Technical State University in Charlotte, North Carolina, and Dr. Muhammad Mian and Jonathan Gibbs from Rust College in Holly Springs, Mississippi. The student and faculty fellows participated in a mix of activities at the DNALC and the Hazen Genome Sequencing Center.

In 2005, we continued our NSF-funded *Greenomes* workshops to train college faculty to use our laboratory- and Internet-based curriculum, bringing students up to date with modern plant research. The objective is to use plant systems to illustrate major concepts of molecular and genomic biology, 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.

Sixty-two faculty participated at weeklong *Greenomes* workshops conducted at San Jose State

University, California (site host Dr. Katy Korsmeyer), Cornell University, Ithaca, New York (site host Dr. Sharon Mitchell); and Virginia Polytechnic Institute and State University, Blacksburg, Virginia (site host Dr. Erin Dolan). We also collaborated with 2004 NSF fellows Dr. Javier Gonzalez-Ramos and Dr. Olga Kopp to conduct regional 3-day plant workshops at at Texas A&M University Agricultural Research and Extension Center in Weslaco and at the Utah Valley State College in Orem. Thirty-one high school and college faculty conducted experiments from the *Greenomes* curriculum.

In 2005 we partnered with Carolina Biological Supply Company (CBSC) to significantly expand our presence at teacher professional meetings. CBSC has distributed DNALC experiment kits to science teachers since 1986, so we were pleased when they offered to provide us free space in the large exhibits they organize for teacher meetings around the United States. Sharing exhibit space with CBSC—and cosponsoring workshops with them—allowed us to reach 381 educators at 11 mini-workshops held at five conventions: the National Science Teacher Association (NSTA) National Convention, Dallas, Texas; the National Association of Biology Teachers (NABT) National Convention, Milwaukee, Wisconsin; the California Science Teacher Association (CSTA) Annual Convention, Palm Springs, California; the Texas Science Teacher Association (CAST) Annual Meeting, Houston; and the National Science Teacher Association (NSTA), regional convention, Nashville, Tennessee.

Cold Spring Harbor Partnership

Since its founding in 1988, the DNALC's interaction with students has been limited to several hours during academic-year field trips or several days during summer DNA camps. In the fall of 2005, we realized a long-held goal of having a sustained relationship with students, when we welcomed our first class of 21 students from neighboring Cold Spring Harbor High School (CSHHS) into the CSH Partnership Program. The full-year course is cotaught by DNALC staff and CSHHS biology teacher Scott Renart. On alternating days, students spend their final two periods at the DNALC doing biochemical experiments or bioinformatics.

Under the program, CSHHS students have the opportunity to access experiment and computer technology developed by the DNALC over the past 20 years with more than $10 million in federal and foundation support. The students conducted a range of experiments in bacterial, plant, animal, and human systems that would typically be found in an upper-level biology elective at a major research university. Students learned principles of gene manipulation by making and analyzing recombinant DNA

Cold Spring Harbor High School students work with Dave Micklos in the computer laboratory. Newly installed graphics depict a DNA sequence from the mitochondrial control region studied in human genetics programs at the DNALC.

CSHHS biology teacher Scott Renart discusses details of a molecular genetics laboratory with a student.

molecules in the bacterium *E. coli*. The class then moved on to study genome analysis in the model plants *Arabidopsis* and corn, to assay for transgenes in genetically modified (GM) food, and to explore newly sequenced genes in rice. In a unit on human genetics, students looked at variations in their own DNA, explored human origins, and performed a population study on the molecular basis of taste and smell. The year ended with the students testing our latest set of experiments using RNAi to silence genes in the nematode worm *C. elegans*.

Genetics as a Model for Whole Learning

Our middle school outreach program, *Genetics as a Model for Whole Learning* (GMWL), continues to offer a variety of age-appropriate activities and experiments designed to introduce 5–7th grade students to genetics. During the academic year, we worked closely with teachers and administrators to develop sequential programs that include in-school instruction by DNALC instructors, field trips to the DNALC, and in-service science training for teachers. Despite lean times for education in Long Island school districts, more than 60 school systems participated in the 2005 program, with 9900 students participating in lab field trips at the DNALC, and 9500 students receiving in-school instruction from DNALC staff.

In-school instruction typically included microscopic examination of cells, observing mutations in *Drosophila*, making DNA models, and studying the genetics of human traits. Armed with the basics of cell biology and genetics, students are then prepared for a culminating field trip to the DNALC. As in years past, the most popular field trip experiences included extracting DNA from bacteria, genetically engineering bacteria with a gene for bioluminescence, touring our interactive exhibit *The Genes We Share,* and solving *The Mystery of Anastasia* computer lab. A new lab, *CSI: Learning Center*, taps into the popular forensics theme of nighttime television. Students bring their enthusiasm for all things *CSI*, and we supply them with the DNA science behind crime scene analysis.

Many of the founding GMWL districts—including Syosset, Jericho, Great Neck, Locust Valley, and Half Hollow Hills—have gone on to develop sequenced genetics instruction at several grade levels taught by their own staff. These districts serve as models for other districts striving to meet new state and national teaching mandates in science. To aid in this effort, we now include information on how our labs align with New York State, Federal, and AAAS standards for science teaching.

Watson School of Biological Sciences

Our collaboration with the Watson School of Biological Sciences provides the CSHL graduate students with a unique teaching opportunity. Rather than the traditional training of graduate students as Teaching Assistants, Watson School students complete a spring rotation working with middle and high school students at the DNALC. During the course of 12 half-day sessions, students work in pairs under the tutelage of seasoned DNALC instructors.

During the first phase of the training, students observe a DNALC instructor deliver a laboratory to a visiting class. Postobservation, the students begin to organize a lesson plan, which integrates their own experience within the context of a specific experiment. The second phase is coteaching, during which each student is responsible for delivering a specific part of the laboratory. The third phase is independent instruction, during which the students work together to present an entire laboratory under the observation of the DNALC instructor. During each phase, students receive oral and written critiques aimed at strengthening their presentation and class management skills. After repeating this learning process at the middle school and high school levels, the students are required to independently teach an additional three lessons of their choice.

Some students opt to travel to a local school district to deliver instruction to several middle school classes. Although the CSHL graduate students are well versed in molecular biology, few have ever attempted to teach these concepts to young students. We believe that the skills required to deliver a successful lab experience to precollege students—engagement, organization, and time management—are the same skills needed to communicate with any audience.

Saturday DNA!

Saturday DNA! continues to involve families in hands-on, minds-on explorations of advances and issues in genetics. Each 2-hour program is designed for children 10–13 years of age (with an accompanying adult) or groups ages 14 through adult (with chaperone for participants under 15). During the year, we presented the "Best of *Saturday DNA!*"—rescheduling previously popular programs such as "RNAi: The Destroyer" and "Food for Thought."

New programs included "Walking Whales and Genetics Tales," which explored the ancient relationship between whales and hippos. Although these two animals are physically very different, participants used sequence analysis to show their close genetic connections. "Stem Cells: When Does Life Begin?" broke free of the traditional lab setting, allowing participants to explore the science behind stem cell research. Participants then used their newfound knowledge to defend a point of view in a debate on the social and ethical issues of stem cell research.

Staff and Interns

The management of the DNALC was significantly bolstered in 2005 with a number of high-level appointments. Uwe Hilgert was named assistant director, a post that had been vacant for several years. After joining the DNALC in November 2000, Uwe quickly took over bioinformatics instruction of a Howard Hughes Medical Institute program. He received a Ph.D. from the Max-Planck Institute for Plant Breeding in Cologne and conducted postdoctoral research at the University of Arizona. Uwe is responsible for managing the instructional staff and interns, for overseeing laboratory operations here and at DNALC *West*, and for organizing workshops and presentations at professional meetings.

John Connolly took the reins as lead producer for *G2C Online*. John recently received his Ph.D. in neuropsychology from Trinity College, Dublin. His background in neuroscience and sociological research will facilitate his work with the broad range of *G2C* collaborators. Bruce Nash and Greg Chin were specifically recruited to jump-start our effort to popularize *C. elegans* and RNAi in biology instruction. Bruce received his Ph.D. in medical and molecular genetics at the University of Toronto. He did postdoctoral research on cell division in *C. elegans* at the University of Oregon. Greg received his Ph.D. in developmental biology from Stanford University, followed by a postdoctoral period at the DNAX Research Institute and teaching at the University of California, Los Angeles.

Bruce Nash, Uwe Hilgert, John Connolly, and Greg Chin

Laura Johns, Stacy Leotta, and David Gundaker

We were also pleased when Danielle Kearns-Sixsmith returned part-time to the DNALC to take on evaluation of the *G2C* site as part of her doctoral research in education at the University of Phoenix, Arizona. Formerly an education manager at the DNALC, she has a B.S. in biological sciences and M.S. degrees in secondary science education and educational leadership. David Gundaker joined the full-time staff as laboratory instructor. With a master's degree in teaching and experience in middle schools in Fort Collins, Colorado and on Long Island, Dave brings depth to the instructional group. In the fall, Stacy Leotta became a part-time member of the DNALC administrative staff. Laura Johns stepped in as part-time lab instructor, filling a gap when Elna Carrasco left on maternity leave. Laura has a bachelor's degree in genetic engineering, has done graduate work in marine chemistry, and has experience cloning ion channels at a small biotech company. Another new addition to the DNALC "family" came in August when middle school educator Erin Maroney was married to James McKechnie.

During the year, we bid farewell to five staff members at the DNALC: Judy Cumella-Korabik, Dr. Shirley Chan, Dr. Tom Bubulya, Dr. Craig Hinkley, and Tracy Behar. Program Manager since 1993, Judy instituted many of the successful administrative methods that helped the DNALC prosper over the last decade. Shirley was the first multimedia producer hired by the embryonic *Biomedia* Group in 1997 and did much of the editorial work for four major Internet sites: *DNA from the Beginning*, *DNA Interactive (DNAi)*, *Your Genes, Your Health (YGYH),* and *Inside Cancer*. She is now director of interactive media at Anatomical Travelogue in Manhattan. After helping to significantly reorganize the DNALC research and development component, Tom and Craig left their positions as scientific managers. Tom moved with his family to Dayton, Ohio, where he is an instructor in the Department of Biological Sciences at Wright State University. Craig and Tom worked as a team to develop and test new instructional protocols. Craig joined the biology department of Kingsborough Community College, CUNY. Tracy Behar left her position as laboratory instructor to teach science at Elwood High School.

High school interns continued to provide key support for our teaching labs, and several carried out independent research projects under the direction of DNALC and CSHL staff. Rachel Stephan (Kings Park High School), a new addition to the intern team, worked with Bruce Nash on an independent research project investigating the effect of known cancer genes on RNA interference.

Our sequencing service continued to grow with the help of college interns Alina Duvall (Hofstra University), Jennifer Aiello (C.W. Post), and newcomer Alexandra Sloane (Loyola College, Maryland). To process the growing number of requests, we now collaborate with Dr. Dick McCombie's group at the Woodbury Genome Center, where high-throughput capillary sequencing has greatly reduced the turn-around time.

Joining the intern program in 2005 were Matthew Giambrone (Walt Whitman High School), Ian Hogg (Friends Academy), Matthew Levy (Kings Park High School), Ronnie Morasse (Plainedge High School), Margarita Varer (Huntington High School), Nick Wilken (Kings Park High School), and Janice Yong (Kings Park High School).

Several interns returned from college to assist with summer workshops: Benjamin Blonde (Amherst College), Bryn Donovan (University of Delaware), Michelle Louie (George Washington University), Marie Mizuno (Binghamton University), and Alex Witkowski (SUNY, Albany).

In August, we bid farewell to the following interns as they began their freshman year at college: Regina Hu (Northport High School) is studying professional pharmacy at St. John's University; Kimberly Izzo (Kings Park High School) is pursuing a double major in biology and vocal performance at Indiana University; Andrew Langer (John H. Glenn High School, Elwood) is studying computer engineering at SUNY Binghamton; and Elena Melius (Oyster Bay High School) is studying business at Washington University in St. Louis.

David A. Micklos
Executive Director

January 8	*Saturday DNA!*, "A Bug's Life," DNALC
January 11	Site visit and museum tour for Watson School of Biological Sciences Students
February 3	Site visit and tour by Jim Parrish, Roger Phillips, Lisa Darmo, and Lawrence Wallace of Carolina Biological
February 12	*Saturday DNA!*, "CSI: Learning Center" and "Animal Models: Showing Off Genes," DNALC
February 15	West Side School Lecture
March 3	Site visit by Xiao-ya Chen, Ai-Zhen Zhang, Yu-lian Wu, Qi Qian, Rui Ming, and Gengxin Chen of Zhejiang University, China
March 9	Genetics Education Program for High School Biology Teachers
March 9	Site visit by Robert Ballard, Renea Hardwick, Kathy Kegley, and John Cummings of Clemson University, South Carolina DNALC
March 10–11	*G2C* Advisory Panel Meeting with Robert Ballard, Renea Hardwick, John Cummings, John Coffey, Mary Colvard, Seth Grant, Louise Gruenberg, Russ Hodge, Pauline Lowrie, Joseph D. McInerney, Joseph Novak, Sarah Robinson, Arati Singh, and Bronwyn Terrill, the Banbury Center
March 11	Site visit by WLIW (Channel 21); filming for "Ticket" about the cultural and public offerings at CSHL
March 15	Site visit by Neil Sandler and Darlene Backelman, Symphony Capital, New York
March 15–16	Genetics Education Program for High School Biology Teachers
March 21	Site visit by Jean Caron, Abby Demars, Jessica Powell, Gary Johnson, Irv Schloss, and Dennis Curran of DNA Epicenter, New London, Connecticut
March 22	Genetics Education Program for High School Biology Teachers
March 30–April 1	NSTA National Meeting, Dallas, Texas
March 31	West Side School Lecture
April 2	*Saturday DNA!*, "Food for Thought" and "When Dinosaurs Roamed the Earth," DNALC
April 4	*Great Moments in DNA Science* Honors Student Seminar, CSHL
April 5	Genetics Education Program for High School Biology Teachers
April 9	*Saturday DNA!*, "It's Not Just Scientists" and "Stem Cells: When Does Life Begin?," DNALC
April 11	*Great Moments in DNA Science* Honors Student Seminar, CSHL
April 14	Site visit and museum tour for Ed Blaskey, Commerce Bank, Melville, New York
April 18	*Great Moments in DNA Science* Honors Student Seminar, CSHL
April 22	Site visit and tour by Jim Chinitz, VP Enzo BioChem, Farmingdale, New York
April 29	Site visit by Robert Frehse, Executive Director of The William Randolph Hearst Foundation, Manhattan, New York
May 3	Site visit by Tahashi Hirata, Chairman, and Hirofumi Nakano, Research Fellow of Kyowa Hakko Biofrontier Laboratories, Tokyo, Japan
May 9–11	*Human DNA Variation, Populations, and Medicine* Meeting for Novartis Oncology
May 14	*Saturday DNA!*, "Walking Whales and Genetic Tales" and "Is There a Neanderthal in Your Family Tree?," DNALC
May 23	Site visit and tour by Jon Cooper, Suffolk County Legislature, Huntington, New York
May 24	Site visit and tour by Janet Jones, Director of Community Affairs at Verizon, Garden City, New York
May 25	Huntington High School Young Professional Group Meeting hosted by Public Affairs Office
June 2	Site visit by Kidgie Williams, diplomats and family members of UN representatives, hospitality committee for United Nations Delegations, Inc., Manhattan, New York
June 6–8	NSF *Plant Molecular Genetics and Genomics* Workshop, Orem, Texas
June 11	*Saturday DNA!*, "The Fluid of Life" and "RNAi: The Destroyer," DNALC
June 15–17	NSF *Plant Molecular Genetics and Genomics* Workshop, Weslaco, Texas
June 20–24	NSF *Greenomes* Workshop, San José, California
June 21	Site visit by Joe Novak, Institute of Human and Machine Cognition (IHMC), Florida; Laura Maitland, AP Psychology Consultant; and Mary Colvard, Education Consultant
June 21	Site visit by Tom Dolan, Principal; Scott Renart, Science Teacher; and 70 students and parents from Cold Spring Harbor High School
June 25–July 9	NSF Faculty Fellowship, Robert Wheeler, Pine Creek High School, Colorado Springs
June 26–July 16	Site visit by Ai Hoon Soh, Meridian Junior College, and Peow Ming Foo, Victoria Junior College, Singapore

June 27–June 30	*Fun with DNA* Workshop, DNALC
	Fun with DNA Workshop, DNALC *West*
	World of Enzymes Workshop, DNALC
June 27–July 2	NSF Faculty Fellowship, Dr. Debra Burhans, Canisius College, Buffalo, New York
June 27–July1	Site visit by Judy Holwell, University of Georgia
July 3–16	USDA Faculty Fellowship, Charlie Gutierrez, John H. Reagan High School, Austin, Texas
July 5–8	*Fun with DNA* Workshop, DNALC
	Green Genes Workshop, DNALC *West*
	Green Genes Workshop, DNALC
July 7	Site visit by William Fair, VP, Monique Salazar, and Dun Wang from East River Science Park/NYC Economic Development Corporation, Manhattan, New York
July 7	Site visit by Christopher Richie, National Institute for Diabetes and Digestive and Kidney Diseases, NIH
July 8	G2C *Online* Interview with Jeffrey Lieberman, Columbia University, New York
July 10	NSF Faculty Fellowship, Mary Smith and Timothy Raines, North Carolina State University, Greensboro, North Carolina; and Muhammad Mian and Jonathan Gibbs, Rust College, Holly Springs, Mississippi
July 11	G2C *Online* Interview with David Porteous, University of Edinburgh, United Kingdom
July 11–15	*World of Enzymes* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
July 12	G2C *Online* Interview with Daniel Weinberger, National Institute of Mental Health, Maryland
July 13	G2C *Online* Interview with Pat Levitt, Vanderbilt University, Tennessee
July 18	G2C *Online* Interview with Sukhi Shergill, Kings College London, United Kingdom
July 18–22	*Fun with DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
July 25–29	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
July 25–29	Bioinformatics in the Classroom Workshop, St. Paul, Minnesota
July 26	Site visit by Frank Posillico, President of Rose Racanelli Real Estate, Dolan DNALC Corporate Advisory Board member
July 27	Site visit and tour by Kaoru Ushijima, Director, Museum Management Division, Chiba Prefectural Government, Tashiro, Director/Curator of the Japan Science Foundation, and translator, Chiba, Japan
August 1	G2C *Online* interview with Karim Nader, McGill University, Canada
August 1	Site visit by Arthur Spiro, CSHL Trustee, and Cary and Lisa Kravat of Kravat Fabrics
August 1–5	NSF Plant Genetics Workshop, Ithaca, New York
August 1–5	*Fun with DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
	Genetic Horizons Workshop, DNALC
August 3	G2C *Online* interview with Earl Miller, Massachusetts Institute of Technology
August 3	G2C *Online* interview with Howard Eichenbaum, Boston University, Massachusetts
August 3	Site visit by Doug Sipp, Miki Murase, Naoki Nambi, and Toshitsugu Hirauchi of the Ricken Center for Developmental Biology, Japan
August 4	G2C *Online* interview and site visit by Seth Grant, Wellcome Trust Sanger Institute, Cambridgeshire, United Kingdom, and Joseph Novak, Institute of Human and Machine Cognition (IHMC), Florida
August 5	G2C *Online* interview with Ron Davis, Baylor College of Medicine, Texas
August 8-12	*Fun with DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
August 8–12	NSF Plant Genetics Workshop, Blacksburg, Virginia
August 10–12	Site visit by Laura Maitland, AP Psychology consultant, New York
August 15–19	Genomic Biology, PCR, and Bioinformatics Workshop, Aspen, Colorado
August 15–19	*Fun with DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Genetic Horizons Workshop, DNALC
August 16–19	Site visit by Laura Maitland, AP Psychology consultant, New York
August 22–26	*Fun with DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
August 25	Site visit and tour by the Champalimaud Foundation, Lisbon, Portugal

August 29–Sept. 2	*World of Enzymes* Workshop, DNALC
August 29–31	Site visit by Laura Maitland, AP Psychology consultant, New York
August 30	Site visit and tour by Marianne Carlton, Director of Internal Communications, and James Cuniglio, Communications Coordinator, Arrow Electronics, Melville, New York
September 12	Public Meeting for Long Island ALS families on ALS research
September 13	Cold Spring Harbor High School Partnership Program commences
September 14	Site visit by Brad Stefanoni, Director of Business Partnerships, and Lisa Blair, Science Education Center Director from Southeast Kansas Education Service Center, Greenbush
September 28	Site visit by Jane Block, former Dolan DNALC Corporate Advisory Board member
October 5–9	NABT National Convention, Milwaukee, Wisconsin
October 7	*G2C Online* Interview with Davie Van Vactor, Harvard Medical School, Massachusetts
October 15	*Saturday DNA!*, "The Building Block of Life" and "Food for Thought," DNALC
October 17	"Building Family Programs" panel presentation by Long Island Museum Association
October 19	Site visit by Rob Dickstein of Pall Corporation, Dolan DNALC Corporate Advisory Board member
October 24	West Side School Lecture
October 25–29	ASHG Convention, Salt Lake City, Utah
October 27–29	CAST Convention (Texas Science Teachers), Houston
October 27–29	CSTA Convention (California Science Teachers), Palm Springs
October 30	Site visit by Long Island Association (LIA)
November 2	Site visit by Christine Leonardis, Manager Business Development from Long Island Works Coalition
November 7	Site visit by Chris Barlow-Stewart from the University of Sydney, Australia
November 8–9	STANYS Conference, Ellenville, New York
November 9	Excellence in Science Award Reception for the American Association of University Women, Huntington Branch
November 11	Site visit by Scott Livingston, Managing Partner from Axiom Capital Management, Manhattan, New York
November 12	*Saturday DNA!*, "CSI: Learning Center" and "Is There a Neandertal in Your Family Tree?," DNALC
November 16	Site visit by members of the Three Harbors Garden Club, Chapter of Garden Club of America, Cold Spring Harbor area
November 17	Site visit by Ed Guiliano, President of New York Institute of Technology, and Lexa Logue, Provost
Nov. 21–Dec. 9	Teacher Training Workshop, National Institute of Education and Singapore Science Center, Singapore
November 28	Site visit by Dori and Peter Tilles (Doris M. and Peter S. Tilles Foundation) and Pat and Edward Travaglianti (CSHL Trustee and President of Commerce Bank of Long Island)
Nov. 28–Dec. 10	Teacher training at the DNALC, Tan Chwee Li, Fengshan Primary School; Ngian Bang Yee, Seng Kang Primary School; Chua Yen Ling, Monfort Junior School; and Yean Sok Kheng, Yio Chu Kang Primary School; Singapore
December 1–3	NSTA Southern Area Convention, Nashville, Tennessee
December 10	*Saturday DNA!*, "The Mystery of Anastasia Romanov" and "RNAi: The Destroyer," DNALC
December 30	Site visit by Janos Posfai from New England BioLabs, Inc.

Sites of Major Faculty Workshops 1985–2005

Key: *Middle School* **College** High School

State	Site	Year
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	**California State University, Fullerton**	**2000**
	Canada College, Redwood City	1997
	Contra Costa County Office of Education, Pleasant Hill	2002
	Foothill College, Los Altos Hills	**1997**
	Harbor-UCLA Research & Education Institute, Torrance	2003
	Laney College, Oakland	1999
	Lutheran University, Thousand Oaks	1999
	Pierce College, Los Angeles	**1998**
	Salk Institute for Biological Studies, La Jolla	2001
	San Francisco State University	**1991**
	San Jose State University	**2005**
	University of California, Davis	1986
	University of California, Northridge	**1993**
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
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
	Biogen, Cambridge	2002
	Boston University	**1994, 1996**
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	Winsor School, Boston	1987
	Whitehead Institute for Biomedical Research, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
MINNESOTA	University of Minnesota St. Paul, St. Paul	2005
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990, 1991
MISSOURI	Stowers Institute for Medical Research, Kansas City	2002
	Washington University, St. Louis	1989
	Washington University, St. Louis	**1997**
NEW HAMPSHIRE	**New Hampshire Community Technical College, Portsmouth**	**1999**
	St. Paul's School, Concord	1986, 1987
NEVADA	University of Nevada, Reno	1992
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
NEW YORK	Albany High School	1987
	Albany UWE	2004
	Bronx High School of Science	1987

	Columbia University	**1993**
	Cold Spring Harbor High School	1985, 1987
	Cornell University, Ithaca	**2005**
	DeWitt Middle School, Ithaca	*1991, 1993*
	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	1997
	Orchard Park Junior High School	*1991*
	Plainview-Old Bethpage Middle School	*1991*
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Stuyvesant High School	1998–1999
	The Rockefeller University	2003
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Trudeau Institute, Lake Saranac	2001
	Union College, Schenectady	2004
	US Military Academy, West Point	**1996**
	Wheatley School, Old Westbury	1985
NORTH CAROLINA	Center for Health Research, Triangle Park	2003
	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	North Westerville High School	1990
OKLAHOMA	**Oklahoma City Community College**	**2000**
	Oklahoma Medical Research Foundation, Oklahoma City	2001
	Oklahoma School of Science and Math, Oklahoma City	1994
OREGON	Kaiser Permanente-Center for Health Research, Portland	2003
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
SOUTH CAROLINA	**Clemson University, Clemson**	**2004**
	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	**1988**
TEXAS	Austin Community College-Rio Grande Campus	2000
	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Southwest Foundation for Biomedical Research, San Antonio	2002
	Taft High School, San Antonio	1991
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999, 2004**
UTAH	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998, 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
	Virginia Polytechnic Institute and State University, Blacksburg	**2005**
WASHINGTON	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001
	University of Washington, Seattle	**1993, 1998**
WASHINGTON, D.C	**Howard University**	**1992, 1996**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Blood Center of Southeastern Wisconsin, Milwaukee	2003
	Madison Area Technical College	**1999**
	Marquette University, Milwaukee	1986, 1987
	University of Wisconsin, Madison	1988, 1989
	University of Wisconsin, Madison	**2004**
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	2001–2005
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995
	Uppsala University, Uppsala	2004

COLD SPRING HARBOR LABORATORY PRESS

2005 PUBLICATIONS

SERIALS

Genes and Development, Vol. 19, 1–3128 (www.genesdev.org)

Genome Research, Vol. 15, 1–1858 (www.genome.org)

Learning and Memory, Vol. 12, 1–664 (www.learnmem.org)

Protein Science, Vol. 14, 1–3188 (www.proteinscience.org)

RNA, Vol. 11, 1–1952 (www.rnajournal.org)

Cold Spring Harbor Symposia in Quantitative Biology, Vol. 69: Epigenetics (www.cshl-symposium.org)

LABORATORY MANUALS

Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, 2005 Edition, David C. Amberg, Daniel J. Burke, and Jeffrey N. Strathern

Protein–Protein Interactions: A Molecular Cloning Manual, 2nd Edition, Erica A. Golemis and Peter D. Adams (eds.)

Basic Methods in Light Microscopy, David Spector and Robert D. Goldman (eds.)

HANDBOOKS

Lab Dynamics: Management Skills for Scientists, by Carl M. Cohen and Suzanne L. Cohen

Laboratory Research Notebook

MONOGRAPHS

RNA World, 3rd Edition, Raymond F. Gesteland, Thomas R. Cech, and John F. Atkins (eds.)

Telomeres, 2nd Edition, Titia de Lange, Vicki Lundblad, and Elizabeth Blackburn (eds.)

The Dog and Its Genome, Elaine A. Ostrander, Urs Giger, and Kerstin Lindblad-Toh (eds.)

Cell Biology of Addiction, Bertha K. Madras, Christine M. Colvis, Jonathan D. Pollock, Joni L. Rutter, David Shurtleff, and Mark von Zostrow (eds.)

GENERAL INTEREST

The Inside Story: DNA to RNA to Protein, Jan Witkowski (ed.)

Landmark Papers in Yeast Biology, Patrick Linder, David Shore, and Michael N. Hall (eds.) (includes CD)

You, Me, and HIV: With Knowledge We Have Hope! by Fran Balkwill and Mic Rolph. English, Zulu, and Afrikaans editions. *Educator's Guide* (in English)

OTHER

CSHL Annual Report 2004: Yearbook Edition

CSHL Annual Report 2004

Banbury Center Annual Report 2004

Watson School of Biological Sciences Annual Report 2004

WEB SITES

www.evolution-textbook.org: Web site to accompany *Evolution* (December 2006 pub date)

www.gastrulation.org: Updated with downloadable figures from *Gastrulation*

www.proteinsandproteomics.org: Updated with release of *Protein–Protein Interactions: A Molecular Cloning Manual, 2nd Edition*

www.rna.cshl.edu: Web site to accompany *RNA World, 3rd Edition*. Includes downloadable chapters from the 2nd edition.

www.you-me-and-hiv.org: Web site to accompany *You, Me, and HIV: With Knowledge We Have Hope!*

A selection of recently published books

The journal publishing program

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

Cold Spring Harbor Laboratory Press is the largest of the five educational divisions of the Laboratory. Continued expansion in recent years has resulted in a staff of 55, offices on the Woodbury campus, marketing operations based in San Diego, California; Oxfordshire, United Kingdom; and Tubingen, Germany; and distributors in Japan, China, Korea, India, and South America.

The Press has a mission to further and financially support the Laboratory's commitment to the advance and spread of scientific knowledge. Our research journals, books, and manuals assist the continuing professional education of working scientists and graduate students, our textbooks help to educate college undergraduates, and our other books delve into the practice, personalities, and history of science and its influence on medicine, business, and social policy. Financially, in 2005, for a second consecutive year, the Press substantially exceeded its budgeted financial goals, with an operating margin of more than $670,000 and income of over $9.75 million. Its total contribution to the Laboratory's economy, including support for institutional overhead and depreciation, was over $1,250,000.

Several achievements underpinned this strong performance. Innovative production management, including the use of off-shore vendors for composition and printing, generated significant cost savings in book and journal publishing. Income from several sources also improved. Against industry trends, institutional subscriptions to the five journals increased by 9%, and the number of site licenses sold increased substantially, assisted by a new contract with the National Science and Technology Library of China on behalf of a consortium of major centers of scientific research. Advertisement and sponsorship sales for all journals improved by more than 10%. The book program released 12 new titles, bringing the total number of books in print by the end of year to over 200. More than 11,800 book and multimedia orders were fulfilled and over 65,000 units shipped. A contract in which Cold-Spring-Harbor-branded protocols were published in the journal *Nature Methods* brought income and valuable book promotion among a wide readership worldwide, and the co-marketing of the online database MolecularCloning.com with *Nature Methods* was also financially advantageous. Fees for the provision of fulfillment and distribution services in the United States for two other publishers generated additional revenue. In addition, BioSupplyNet.com, the online directory of laboratory supplies and suppliers relaunched in 2004, added more than 500 new product categories and improved its revenue contribution by over 13%.

The 12 newly published titles were a mix of manuals, monographs, reference, and history books. The best-seller was *Basic Methods in Microscopy*, a manual by David Spector and Robert Goldman. There were new editions of monographs on telomeres and RNA biology and a manual on protein–protein interactions. A monograph on dogs and their value in gene and genome studies was bathed in the spotlight of widespread press coverage when the sequence of the dog genome was published in December. Sales were soundly supported by the perennial favorites *Molecular Cloning* and *At the Bench*.

A large proportion of our recent books were chosen for review in the professional scientific press, invariably receiving warm words for the quality of the contents and the authority of the authors and editors who created the books. We are extremely fortunate to be able to call on busy scientists of such distinction as participants in our projects.

Appreciation of the publishing program's value was further evident in the expanded use made of journal content. Online readers downloaded articles from the five journals on average 140,000 times per month, 27% more than in the previous year. Over 13,000 journal pages were published, an increase of 9%, representing 1,115 new articles. Many of these papers were highlighted in the world's major news media, including the *New York Times, Wall Street Journal*, and the BBC News. The impact factors of four journals increased significantly, *Genome Research* improving 8% to 10.38. The impact

factor of *Genes & Development*, at 16.385, although slightly down, remained one of the 20 highest impact factors among all primary research journals.

The annual Symposium was published online for the second time. Institutional purchasers now have access to Symposium manuscripts many months ahead of print publication and to other features such as video interviews with speakers at the meeting. Thirteen contracts for new books were signed, including innovative textbooks in human biology, neurobiology, and developmental biology. Ten agreements were signed with foreign publishers for translations into Chinese, Japanese, German, and Korean, and nine contracts were agreed for foreign reprint rights.

Copies of the Gates-Foundation-supported second edition of the book for teenagers, *You, Me, and HIV,* and an associated teacher's guide, were distributed in three provinces of South Africa. A new method of distribution was developed, a cascade strategy involving workshops in which teachers were trained to use our materials in the classroom and also to train other teachers in their use. 50,000 copies in English and 20,000 copies in Afrikaans and Zulu are being made available free through these workshops, which have expanded the horizons of this initiative from publishing to a community education project with the potential of mainstreaming AIDS education in the school curriculum. With the conclusion of the Foundation grant, further financial support for the project is being sought.

Two thirds of Press income now comes from journal publishing, more than 70% of it from subscription sales. Throughout the year, debate continued about the validity of an open-access, funder-supported business model as an alternative to subscriptions for the publication of research results. In this model, reading research results online is free, and a paper's publication is supported by a fee paid on the author's behalf by the organization that funded the research. A growing number of science funding bodies, particularly in Europe, are energetically committed to this alternative, seeing in the Internet the possibility of ending restrictions on public access to newly published science and freeing up, for research, the large sums currently spent purchasing and maintaining journal subscriptions in academic institutions.

Although publishers and scholarly societies have talked of little else in the past several years, the community of working scientists at large is less engaged. Only a small proportion of authors in *Genome Research* chose to make their papers available in this way when the option was introduced for a modest fee in January. Our journal archives stretching back as much as 10 years are freely accessible online and all back issues are made publicly available no later than 12 months after publication (6 months for *Genes & Development* and *Genome Research*). This openly available material is certainly used heavily, and such access has been made possible within the framework of the current subscription model. It is unclear if funding bodies will be prepared to pay a publication fee high enough to sustain a well-regarded, highly selective journal without additional direct grant support or private underwriting. Such journals have the irreducibly fixed cost of a professional editorial staff that ensures first-class peer-review, selection, and distribution of papers.

A direct dialog has begun with some of the activist funding bodies about the real costs of publication and a fee structure that would support the high-quality journals that Cold Spring Harbor and other not-for-profit organizations publish. Meanwhile, the policy of the dominant American funding body, the National Institutes of Health, toward the funder-pays model remains in flux—an important uncertainty, since NIH supports well over half the research that appears in our journals. Not all journals can release papers less than 1 year after publication without risking subscription cancellation. Meanwhile, an effort to create a freely available online archive of NIH-funded papers that relies on grantees' voluntary deposition of prepublication manuscripts has achieved low compliance and NIH may be given more teeth by Congress in 2006. Free release of NIH-supported papers within 6 months may soon become mandated.

Clearly, we are still only at the beginning of a period of major turmoil in the economics and ecology of journal publishing. And with increasing interest on the part of online superstars Google and Amazon in delivering online book content to readers, both as whole volumes and as single chapters, the strategic aspects of science publishing have never been more challenging, or intriguing. If merely distributing the work of our distinguished authors electronically were enough, fulfilling the educational aspects of our mission would be simple. Sustaining the enterprise financially, however, is less straightforward.

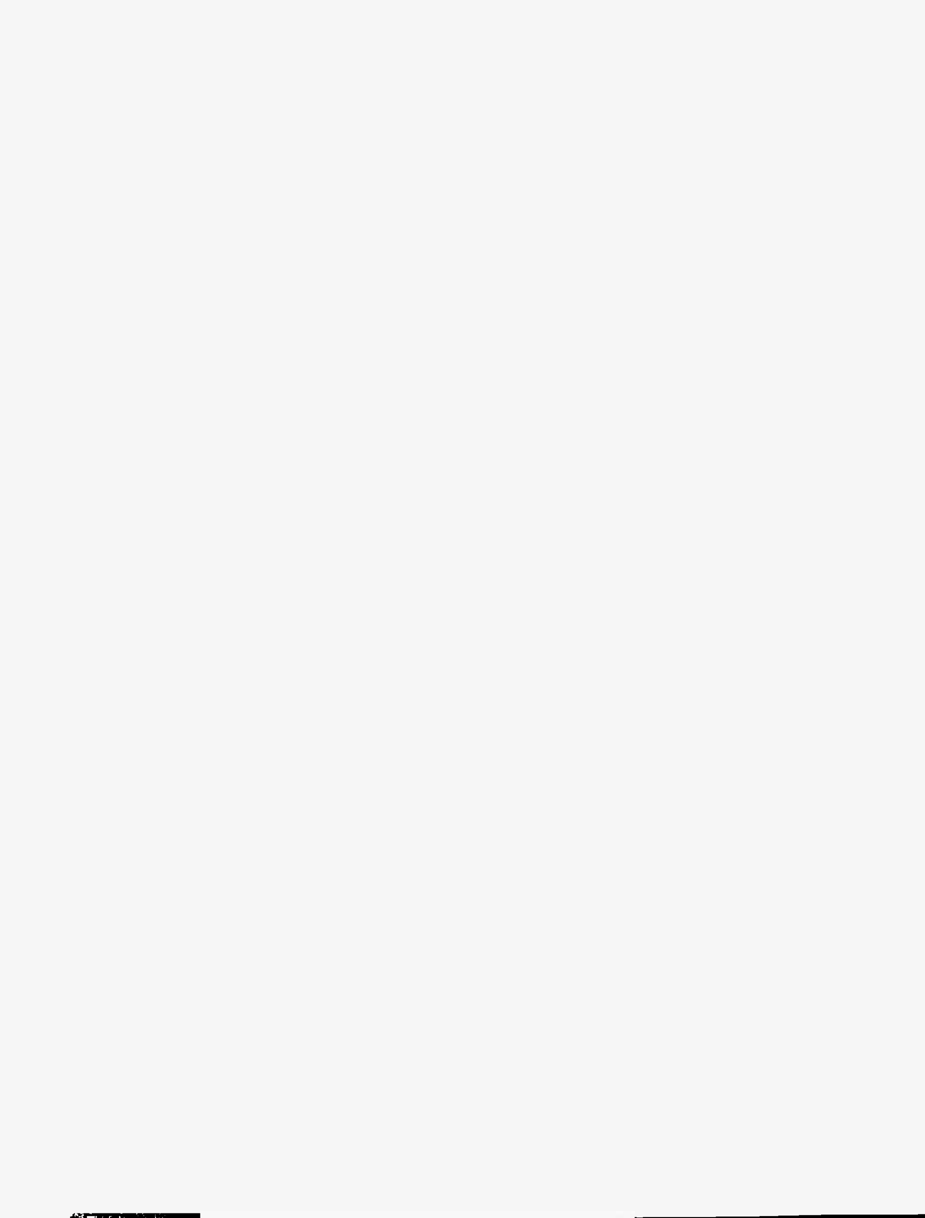
Staff

The staff members of the Press as of December 2005 are listed elsewhere in this volume. Our organization is fortunate to have the support of such a professional and dedicated group of people.

In 2005 we welcomed four new colleagues: Richard Cavalieri, Phillip LoFaso, Wayne Manos, and Nora Ruth. We also said goodbye, thanks, and good luck to Colleen Becker and Kathryn Fitzpatrick. The year's encouraging results were a just reward for the whole staff and the talented managers on whom I depend so much: Jan Argentine, Ingrid Benirschke, Kathy Cirone, David Crotty, Kathryn Fitzpatrick, Alex Gann, Nancy Hodson, Phillip LoFaso, Geraldine Jaitin, Bill Keen, Guy Keyes, Marcie Siconolfi, Linda Sussman, and Denise Weiss. The Press also owes a great deal to the Editors of our successful journals, Terri Grodzicker at *Genes & Development* and Hillary Sussman at *Genome Research*. And it is a pleasure once again to acknowledge the essential contribution that my executive assistant Elizabeth Powers makes to our organization.

John R. Inglis
Executive Director

Press staff members



FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2005
With comparative financial information as of December 31, 2004

		2005	2004
Assets:			
Cash and cash equivalents	$	48,885,313	40,744,417
Accounts receivable:			
Publications		1,206,608	1,308,718
Other		4,528,843	1,179,604
Grants receivable		8,352,217	6,975,455
Contributions receivable, net		47,578,065	12,492,687
Publications inventory		2,979,817	2,945,099
Prepaid expenses and other assets		2,015,019	1,605,707
Investments		232,371,266	211,294,366
Investment in employee residences		5,726,739	4,830,425
Restricted use assets		3,226,538	2,904,923
Land, buildings, and equipment, net		115,851,522	110,757,023
Total assets	$	472,721,947	397,038,424
Liabilities and net assets:			
Liabilities:			
Accounts payable and accrued expenses	$	10,361,413	7,429,657
Notes payable		73,638	107,775
Deferred revenue		4,237,554	3,878,738
Bonds payable		45,200,000	45,200,000
Total liabilities		59,872,605	56,616,170
Net assets:			
Unrestricted		195,431,117	184,397,408
Temporarily restricted		70,289,399	25,419,056
Permanently restricted		147,128,826	130,605,790
Total net assets		412,849,342	340,422,254
Total liabilities and net assets	$	472,721,947	397,038,424

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2005
With summarized financial information for the year ended December 31, 2004

	Unrestricted	Temporarily Restricted	Permanently Restricted	2005 Total	2004 Total
Revenue and other support:					
Public support (contributions and non-Federal grant awards)	$ 22,009,798	49,454,134	12,312,801	83,776,733	30,258,733
Federal grant awards	32,067,800	–	–	32,067,800	29,451,302
Indirect cost allowances	19,558,159	–	–	19,558,159	17,659,458
Program fees	3,583,017	–	–	3,583,017	3,644,734
Publications sales	9,751,069	–	–	9,751,069	9,743,639
Dining services	3,349,002	–	–	3,349,002	3,183,440
Rooms and apartments	2,703,382	–	–	2,703,382	2,632,671
Royalty and licensing fees	2,873,198	–	–	2,873,198	926,913
Investment income (interest and dividends)	4,831,010	–	–	4,831,010	3,134,480
Miscellaneous	652,207	–	–	652,207	514,559
Total revenue	101,378,642	49,454,134	12,312,801	163,145,577	101,149,929
Net assets released from restrictions	4,583,791	(4,583,791)	–	–	–
Total revenue and other support	105,962,433	44,870,343	12,312,801	163,145,577	101,149,929
Expenses:					
Research	57,766,809	–	–	57,766,809	52,049,866
Educational programs	13,207,352	–	–	13,207,352	13,238,766
Publications	9,432,319	–	–	9,432,319	9,380,833
Banbury Center conferences	1,396,425	–	–	1,396,425	1,159,499
Dolan DNA Learning Center programs	1,231,059	–	–	1,231,059	1,611,325
Watson School of Biological Sciences programs	2,848,109	–	–	2,848,109	2,263,518
General and administrative	13,267,025	–	–	13,267,025	12,508,768
Dining services	4,684,031	–	–	4,684,031	4,236,535
Total expenses	103,833,129	–	–	103,833,129	96,449,110
Excess of revenue and other support over expenses	2,129,304	44,870,343	12,312,801	59,312,448	4,700,819
Other changes in net assets:					
Net appreciation in fair value of investments	8,904,405	–	4,210,235	13,114,640	21,815,957
Increase in net assets	11,033,709	44,870,343	16,523,036	72,427,088	26,516,776
Net assets at beginning of year	184,397,408	25,419,056	130,605,790	340,422,254	313,905,478
Net assets at end of year	$ 195,431,117	70,289,399	147,128,826	412,849,342	340,422,254

CONSOLIDATED STATEMENTS OF CASH FLOWS
Year ended December 31, 2005
With comparative financial information for the year ended December 31, 2004

	2005	2004
Cash flows from operating activities:		
Increase in net assets	$ 72,427,088	26,516,776
Adjustments to reconcile increase in net assets to net cash used in operating activities:		
Depreciation and amortization	5,852,278	5,769,622
Net appreciation in fair value of investments	(13,009,353)	(21,746,064)
Contributions restricted for long-term investment	(47,445,268)	(13,812,552)
Changes in assets and liabilities:		
(Increase) decrease in accounts receivable	(3,247,129)	101,178
(Increase) decrease in grants receivable	(1,376,762)	92,932
(Increase) decrease in contributions receivable	(125,496)	79,666
Increase in publications inventory	(34,718)	(738,459)
Increase in prepaid expenses and other assets	(409,312)	(38,716)
Increase in restricted use assets	(321,615)	(195,347)
Increase in accounts payable and accrued expenses	900,672	1,275,293
Increase (decrease) in deferred revenue	358,816	(189,222)
Net cash provided by (used in) operating activities	13,569,201	(2,884,893)
Cash flows from investing activities:		
Capital expenditures	(10,946,777)	(7,273,237)
Proceeds from sales and maturities of investments	134,357,645	113,998,317
Purchases of investments	(142,643,334)	(104,850,454)
Net change in investment in employee residences	(678,172)	(55,591)
Net cash (used in) provided by investing activities	(19,910,638)	1,819,035
Cash flows from financing activities:		
Permanently restricted contributions	12,312,801	1,159,665
Contributions restricted for investment in land, buildings, and equipment	35,132,467	12,652,887
Increase in contributions receivable	(34,959,882)	(1,119,362)
Increase in accounts payable relating to capital expenditures	2,031,084	179,083
Repayment of notes payable	(34,137)	(33,183)
Net cash provided by financing activities	14,482,333	12,839,090
Net increase in cash and cash equivalents	8,140,896	11,773,232
Cash and cash equivalents at beginning of year	40,744,417	28,971,185
Cash and cash equivalents at end of year	$ 48,885,313	40,744,417
Supplemental disclosures:		
Interest paid	$ 1,134,372	780,758

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 2005.

GRANTS January 1–December 31, 2005

COLD SPRING HARBOR LABORATORY

Grantor	Program/Principal Investigator	Duration of Grant	2005 Funding*	
FEDERAL GRANTS				
NATIONAL INSTITUTES OF HEALTH				
Equipment Support	Dr. Spector	04/01/05 03/31/06	$ 452,121	*
	Dr. Stillman	09/01/05 05/31/07	982,065	*
	Dr. Tansey	01/01/05 12/31/05	332,500	*
	Dr. Townsend	06/01/05 05/31/06	1,985,558	*
Program Project Support	Drs. Hannon/Krainer/Lazebnik/Lowe/ S. Muthuswamy/Myers/Spector/ Stenlund/Stillman/Tansey	01/01/97 12/31/06	5,192,593	
	Drs. Lowe/Hannon/S. Muthuswamy	04/01/04 03/31/09	928,197	
	Dr. Stillman-CSHL Cancer Center Core	08/01/05 07/31/10	4,250,000	*
Pioneer Award Support	Dr. Cline	09/30/05 07/31/10	847,501	*
Merit Award Support	Dr. Malinow	05/01/92 04/30/10	654,807	*
	Dr. Tonks	08/01/91 03/31/06	664,827	
Contract Support	Drs. Sebat/Wigler	12/06/05 12/05/06	447,352	*
Research Support	Dr. Brody	07/01/04 04/30/09	377,574	
	Dr. Chklovskii	07/08/04 06/30/09	357,347	
	Dr. Cline	12/01/04 11/30/09	490,018	
	Dr. Cline	03/01/98 03/31/06	492,545	
	Dr. Dubnau	09/15/04 06/30/09	423,428	
	Dr. Hamaguchi	04/01/04 08/31/05	194,925	
	Dr. Hannon	07/01/00 08/31/05	364,425	
	Dr. Hirano	07/01/05 06/30/09	355,950	*
	Dr. Hirano	05/01/96 04/30/08	406,800	
	Dr. Huang	08/01/01 06/30/06	415,000	
	Dr. Huang	07/15/05 04/30/09	391,969	*
	Dr. Joshua-Tor	05/01/01 03/31/06	373,500	
	Dr. Joshua-Tor	02/15/02 01/31/07	373,500	
	Dr. Joshua-Tor	07/01/05 06/30/09	316,973	*
	Dr. Krainer	07/01/03 06/30/07	596,271	
	Dr. Krainer	06/01/01 05/31/06	408,400	
	Dr. Lowe	07/01/04 06/30/09	499,371	
	Drs. Mainen/Brody	09/23/02 08/31/07	376,888	
	Dr. Malinow	04/01/95 02/29/08	571,969	
	Dr. Martienssen	08/01/03 07/31/07	339,000	
	Dr. Mitra	03/04/05 02/28/09	498,976	*

*Includes direct and indirect costs
*New grants awarded in 2005

	Investigator	Start	End	Amount	
	Dr. Mittal	04/01/04	03/31/09	351,887	
	Dr. S. Muthuswamy	03/01/03	02/28/07	377,138	
	Drs. Myers/Herr	03/01/02	02/28/07	359,815	
	Dr. Neuwald	09/30/98	08/31/06	383,788	
	Dr. Sebat	09/30/05	07/31/10	149,997	*
	Dr. Skowronski	04/01/98	04/30/08	660,694	
	Dr. Spector	04/01/90	03/31/07	649,246	
	Dr. Spector	09/01/04	08/31/08	344,113	
	Dr. Stein	09/01/02	03/31/06	524,999	
	Drs. Stenlund/Hernandez	07/01/04	06/30/07	253,912	
	Dr. Stillman	07/01/91	05/31/08	562,401	
	Dr. Svoboda	12/01/03	11/30/08	255,475	
	Dr. Svoboda	12/01/02	11/30/07	225,435	
	Dr. Svoboda	06/01/03	03/31/08	311,966	
	Dr. Svoboda	03/01/04	02/28/09	343,238	
	Dr. Tansey	05/01/03	04/30/07	330,525	
	Dr. Tonks	05/01/97	06/30/05	389,850	
	Dr. Tully	03/01/03	02/29/08	385,631	
	Dr. Tully	08/01/03	05/31/07	381,375	
	Dr. Van Aelst	05/01/03	04/30/08	339,424	
	Dr. Wigler	07/15/98	04/30/07	530,945	
	Dr. Xu	08/01/03	03/31/07	364,425	
	Dr. Xu	08/01/05	03/31/06	85,881	*
	Dr. Zador	01/23/03	12/31/07	519,615	
	Dr. Zhang	09/19/03	08/31/06	705,438	
	Dr. Zhong	07/01/03	06/30/08	347,051	

Research Subcontracts

	Investigator	Start	End	Amount	
NIH/Affymetrix Consortium Agreement	Dr. Stein	08/01/04	05/31/06	118,035	
NIH/Arginox Consortium Agreement	Dr. Enikolopov	02/16/05	02/15/06	44,655	*
NIH/Baylor College of Medicine Consortium Agreement	Dr. Mills	09/01/01	08/31/06	226,656	
NIH/Baylor College of Medicine Consortium Agreement	Dr. S. Muthuswamy	03/01/04	12/31/04	67,800	
NIH/Booz Allen Hamilton caBIG Consortium Agreement	Dr. Stein	08/01/04	02/28/05	264,365	
NIH/Booz Allen Hamilton caBIG Consortium Agreement	Dr. Stein	09/01/04	08/31/06	200,209	*
NIH/Caltech Consortium Agreement	Dr. Stein	09/01/03	08/31/08	729,272	*
NIH/Caltech Consortium Agreement	Dr. Svoboda	04/04/03	02/29/08	148,391	
NIH/Columbia University Consortium Agreement	Dr. Lowe	09/30/00	01/31/06	533,621	
NIH/Evanston Northwestern Consortium Agreement	Dr. Zhang	07/01/03	06/30/06	132,261	
NIH/Massachusetts Institute of Technology Consortium Agreement	Dr. Stein	09/15/04	08/31/06	199,709	
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Mittal	04/01/04	03/31/09	136,686	
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Van Aelst	07/10/03	06/30/08	336,324	
NIH/Nanoprobes, Inc.	Dr. Spector	10/15/05	04/16/06	17,053	*
NIH/Rutgers University Consortium Agreement	Dr. Mitra	12/08/04	11/30/08	140,504	
NIH/Stony Brook University	Dr. McCombie	11/01/05	08/31/06	55,000	*
NIH/Washington University Consortium Agreement	Dr. Stein	09/01/03	08/31/08	56,199	
NIH/Washington University Consortium Agreement	Dr. Stein	11/01/03	10/31/06	56,199	

'Includes direct and indirect costs
*New grants awarded in 2005

NIH/Weill Cornell Medical Center Consortium Agreement	Dr. Mitra	02/01/04	01/31/06	46,711
NIH/University of Wisconsin Consortium Agreement	Dr. Stein	01/01/04	12/31/07	198,171
Fellowship Support	Dr. Lucito	06/01/02	05/31/07	143,473
	Dr. Weimer	07/01/05	06/30/08	49,928 *
	Dr. Wilbrecht	07/01/05	06/30/08	43,976 *
Course Support	Cellular Biology of Addiction	07/15/05	06/30/08	46,250 *
	Cell and Developmental Biology of *Xenopus*	04/01/93	03/31/09	20,023
	DNA Microarray Applications	05/01/99	04/30/07	57,072
	Neurobiology of *Drosophila*	07/01/01	06/30/06	46,189
	Advanced Techniques in Molecular Neuroscience	07/01/01	06/30/06	60,990
	Imaging Structure and Function in the Nervous System	07/01/01	06/30/06	53,348
	C. elegans	08/01/98	07/31/06	71,736
	Proteomics	07/01/03	06/30/06	68,483
	Advanced Bioinformatics	09/01/05	08/31/06	52,000
	Computational and Comparative Genomics	09/01/05	08/31/07	47,605
	Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging	07/01/98	08/31/10	77,962
Meeting Support	Learning and Memory	2005		6,627 *
	The Ubiquitin Family	2005		9,000 *
	Telomeres and Telomerase	2005		22,275 *
	The Biology of Genomes	2005		30,470 *
	Protein Phosphorylation and Cell Signaling	2005		7,000 *
	70th Symposium: Molecular Approaches to Controlling Cancer	2005		5,000 *
	Eukaryotic mRNA Processing	2005		11,373 *
	Mechanisms of Eukaryotic Transcription	2005		8,000 *
	Eukaryotic DNA Replication	2005		5,229 *
	Microbial Pathogenesis and Host Response	2005		13,386 *
	Programmed Cell Death	2005		12,000 *
	Neurobiology of *Drosophila*	2005		23,000 *
	Genome Informatics	2005		22,000 *
	Rat Genomics and Models	2005		15,000 *

NATIONAL SCIENCE FOUNDATION

Multiple Project Award Support	Dr. Jackson	07/01/05	06/30/10	870,780 *
	Dr. McCombie	09/01/03	08/31/06	1,312,408
	Dr. Stein	12/15/03	11/30/07	2,407,499
Research Support	Dr. Hannon	09/01/04	02/28/06	72,263
	Dr. Jackson	03/01/04	02/28/07	145,000
	Dr. Lukowitz	04/01/05	03/31/08	116,770 *
	Dr. Martienssen	08/31/05	02/28/07	100,000 *
	Drs. McCombie/Jackson/Myers	06/15/04	11/30/05	71,984
	Dr. Stein	09/01/03	08/31/06	895,615
	Dr. Timmermans	09/01/03	08/31/06	123,802
	Dr. Van Aelst	08/15/05	07/31/08	131,112 *
	Dr. Ware	09/01/03	08/31/08	553,484

·Includes direct and indirect costs
*New grants awarded in 2005

Research Subcontract Support				
NSF/National Center for Genome Research Consortium Agreement	Dr. Stein	09/01/05	08/31/08	54,497 *
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	10/01/04	09/30/09	256,404
NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	10/01/04	09/30/09	198,945
NSF/University of Arizona Consortium Agreement	Dr. Stein	10/01/03	09/30/07	254,320
NSF/University of California–Berkeley Consortium Agreement	Drs. Jackson/Martienssen	10/01/01	09/30/06	292,580
NSF/University of Florida Consortium Agreement	Dr. Martienssen	09/15/05	08/31/07	50,747 *
NSF/University of Georgia Consortium Agreement	Dr. Timmermans	09/01/03	12/31/05	85,279
NSF/University of Washington Consortium Agreement	Dr. Martienssen	09/01/05	08/31/10	249,080 *
Undergraduate Training Support	URPs: REU Site in Bioinformatics and Computational Biology	04/15/05	03/31/06	107,350 *
Meeting Support	Learning and Memory	2005		8,000 *
	Telomeres and Telomerase	2005		4,000 *
	Eukaryotic mRNA Processing	2005		5,000 *
	Eukaryotic DNA Replication	2005		5,000 *
	Neurobiology of *Drosophila*	2005		10,000 *
Course Support	Advanced Bacterial Genetics	07/15/04	06/30/06	73,721
	Advanced Techniques in Plant Science	07/01/03	06/30/06	81,271
	Cell and Developmental Biology of *Xenopus*	09/01/98	08/31/06	23,503
	The Gramene Community Curation Network	01/15/03	12/31/06	241,616

UNITED STATES DEPARTMENT OF AGRICULTURE

Research Support	Dr. Jackson	09/01/03	08/31/06	76,802
	Dr. Jackson	09/01/05	08/31/08	115,187 *
	Dr. McCombie	09/15/04	09/14/09	37,812
	Drs. McCombie/Martienssen	02/01/04	01/31/07	202,567
	Dr. Stein	09/22/03	09/14/08	740,591
Research Subcontract Support				
USDA/Oregon State University Consortium Agreement	Dr. Martienssen	12/01/04	11/30/07	13,023

UNITED STATES DEPARTMENT OF THE ARMY

Research Support	Dr. Hannon	04/01/02	03/31/06	769,317
	Drs. Kamalakaran/Dubnau	11/16/05	11/15/06	100,000 *
	Drs. Lucito/Wigler	02/01/05	01/31/07	894,579 *
	Dr. Mainen	04/01/04	03/31/06	101,522
	Drs. Wigler/Lucito	04/01/04	03/31/06	2,266,100
	Dr. Zhong	12/15/04	12/14/08	334,511
Fellowship Support	T. Haire	10/01/04	12/31/06	46,191
	A. Lucs	04/01/03	03/31/06	34,108

*Includes direct and indirect costs
*New grants awarded in 2005

	M. Moore	08/16/04	08/15/06	33,694
	E. Murchison	02/16/05	02/15/08	30,000 *
	S. Nunez	02/27/04	02/28/07	30,469
	A. Rosenberg	02/28/05	02/28/08	30,000 *
	D. Siolas	07/01/04	06/30/06	39,248
	K. Siddiqui	04/01/03	03/31/06	36,015
	Dr. Smith	04/01/04	03/31/07	122,984
	Dr. Xiang	06/01/03	05/31/06	58,664
Research Subcontract Support				
U.S. Army/New York University Consortium Agreement	Dr. Lucito	09/27/04	09/26/09	119,785
MISCELLANEOUS				
Equipment Support				
Breast Cancer Help, Inc.	Dr. Stillman	2005		20,000
Breast Cancer Help, Inc./West Islip Youth Enrichment	Dr. Stillman	2005		1,553 *
Program Project Support				
DART Neurogenomics Project	Drs. Tully/Cline/Dubnau/Henry/Mitra/Zhang/Zhong	10/01/04	09/30/07	4,420,060
The Thomas Hartman Foundation for Parkinson Research Partnership	Drs. Cline/Enikolopov/Mainen/Sebat/Tonks/Tully	05/01/05	04/30/10	1,200,000 *
The Leukemia & Lymphoma Society	Dr. Lowe	10/01/03	09/30/08	1,490,451
The Simons Foundation	Dr. Wigler	06/01/06	05/31/08	4,792,700 *
Research Support				
American Cancer Society	Dr. Wigler	01/01/96	12/31/05	10,000
American Cancer Society	Dr. Wigler	01/01/01	12/31/05	70,000
Arginox, Inc.	Dr. Enikolopov	02/01/05	01/31/06	147,345 *
Benefit for the Brain	Dr. Cline	01/01/05	12/31/05	185,648 *
Breast Cancer Research Foundation	Dr. Wigler	10/01/05	09/30/06	250,000 *
Lavinia & Landon Clay	Dr. Brody	12/01/05	11/30/06	200,000 *
CSH Main Street Association	Dr. Powers	06/01/05	05/31/06	8,000 *
Coyle-DeVita	Dr. Powers	07/01/05	06/30/06	25,000 *
DeMatteis Family Foundation	Dr. Powers	10/01/04	09/30/06	450,000 *
Den Haag Foundation	Dr. Stillman	07/01/03	12/31/05	206,400
Diaz Estate	Dr. Stillman	01/01/05	12/31/05	158,591 *
Dunkin Brands	Dr. Powers	11/01/05	10/31/06	5,000 *
ELJI/SUSB Consortium Agreement	Dr. Huang	01/15/04	01/14/07	79,420
Eppley Foundation	Dr. Enikolopov	05/01/05	04/30/06	28,000 *
Find A Cure Today (F.A.C.T.)	Dr. S. Muthuswamy	07/01/03	07/31/06	37,595
Samuel & Hillary Fox	Drs. S. Muthuswamy/Hicks	07/01/05	06/30/06	2,225 *
FSMA	Dr. Krainer	07/15/04	07/14/05	13,333
Gerber Foundation	Dr. Hatchwell	01/01/05	07/31/06	83,300 *
Irving Hansen Foundation	Dr. Tansey	08/01/04	07/31/05	49,413
Harrison's Heart Foundation	Dr. Hatchwell	12/21/04	12/31/05	72,500 *
Ira Hazan	Dr. Enikolopov	12/01/03	11/30/05	200,000 *
James R. Hudson, Jr.	Dr. Hannon	06/01/05	05/31/06	50,004 *
Human Frontier of Science Program Organization (HFSPO)	Dr. Spector	06/01/03	05/31/06	113,815
Human Frontier of Science Program Organization (HFSPO)	Dr. Mitra	06/15/03	06/14/06	96,500

·Includes direct and indirect costs
*New grants awarded in 2005

Town of Islip Breast Cancer Coalition	Dr. Powers	08/01/05	07/31/06	5,000	*
Karches Foundation	Dr. Wigler	07/01/05	06/30/10	439,388	*
Kids Care	Dr. Powers	04/01/05	03/31/06	3,704	*
The Forrest & Francis Lattner Foundation in honor of Andrew Harris	Dr. Sebat	12/16/05	12/16/07	500,000	*
The Charles Leach Foundation	Dr. Enikolopov	01/01/05	12/31/05	10,000	*
Lehman Brothers Foundation, Inc.	Dr. Powers	11/01/05	10/31/06	25,000	*
LI 2-Day Walk to Fight Breast Cancer	Dr. S. Muthuswamy	08/01/04	07/31/06	25,000	
LIABC (Long Islanders Against Breast Cancer)	Dr. Wigler	01/01/00	12/31/06	46,027	*
Long Beach Breast Cancer Coalition	Dr. S. Muthuswamy	07/15/04	07/14/06	2,000	
Manhasset Women's Coalition Against Breast Cancer	Dr. Powers	05/01/05	04/30/06	20,000	*
March of Dimes	Dr. Huang	06/01/04	05/31/07	95,409	
March of Dimes	Dr. Mills	06/01/03	05/31/06	68,686	
The G. Harold & Leila Y. Mathers Charitable Trust	Dr. Zador	07/01/03	06/30/06	170,275	
The Elizabeth McFarland Breast Cancer Fund	Dr. Wigler	03/01/04	02/28/06	35,001	*
Michael's Haven	Dr. Lowe	02/01/05	01/31/06	2,000	*
Miracle Foundation	Dr. Wigler	01/01/05	12/31/05	100,000	*
Louis Morin Charitable Trust	Dr. Spector	12/01/05	11/30/06	75,000	*
NAAR	Dr. Van Aelst	07/01/05	06/30/07	60,000	*
NARSAD	Dr. Mainen	09/15/04	09/14/06	50,795	
NARSAD	Dr. Enikolopov	09/15/04	09/14/05	50,000	
Manyu Ogale	Dr. Wigler	07/01/05	06/30/06	50,000	*
Omidyar Foundation	Drs. S. Muthuswamy/Powers	07/01/05	06/30/06	200,000	*
Open Biosystems	Dr. Hannon	06/01/05	05/31/07	83,300	*
Philip Morris USA	Dr. Mittal	07/01/05	06/30/06	423,750	*
Philips Research	Dr. Zhang	12/01/05	11/30/06	5,000	*
The Fannie E. Rippel Foundation	Drs. Lucito/Tonks	12/01/05	11/30/07	350,000	*
Joan's Legacy: The Joan Scarangelo Foundation to Conquer Lung Cancer	Dr. Mu	11/01/05	10/31/07	50,000	*
The Seraph Foundation	Dr. Enikolopov	01/01/01	09/30/06	74,000	*
SMA Foundation	Dr. Krainer	06/01/04	05/31/07	65,936	
Theodore & Vada Stanley Foundation Schizophrenia Research	Dr. Watson	2005		5,000,000	*
Strelkowski Estate	Dr. Stillman	2005		12,500	*
Waldbaum's Foundation	Dr. Wigler	03/01/05	02/28/05	10,000	*
WALK for Women Breast Cancer Fund	Dr. S. Muthuswamy	2005		6,390	*
West Islip Breast Cancer Coalition of LI, Inc.	Dr. Wigler	11/01/05	10/31/06	10,000	*
Women In Science Program	Dr. Stillman	01/01/05	12/31/05	30,342	*

Fellowship Support

Rita Allen Foundation	Dr. S. Muthuswamy	08/01/04	07/31/05	50,000	
Arnold and Mabel Beckman Foundation	Dr. Dubnau	09/01/05	08/31/08	88,000	*
Burroughs Wellcome	Dr. Karpova	09/01/05	08/31/07	58,000	*
Burroughs Wellcome	Dr. Zito	08/01/04	06/30/06	60,200	
Jane Coffin Childs Foundation	Dr. Rivas	07/01/04	06/30/07	43,500	
Cody Center/Stony Brook University	Dr. Enikolopov	04/01/05	03/31/06	50,000	*
Cody Center/Stony Brook University	Dr. Hatchwell	10/01/04	09/30/07	40,000	*
CSHL Association Fellowship	Dr. Boivin	2005		51,919	*
CSHL Association Fellowship	Dr. Felsen	2005		51,919	*
CSHL Association Fellowship	Dr. Gallavotti	2005		51,919	*
CSHL Association Fellowship	Dr. Lima	2005		51,919	*

•Includes direct and indirect costs
*New grants awarded in 2005

CSHL Association Fellowship	Dr. Nogueira	2005		49,664 *
Damon Runyon Cancer Research Foundation	Dr. Hu	08/01/05	07/31/08	45,000 *
Damon Runyon Cancer Research Foundation	Dr. Zu	04/01/03	03/31/06	45,000 *
DFG German Science Foundation	Dr. Bayer	09/01/05	08/31/07	13,450 *
European Molecular Biology Organization (EMBO) Long-term Fellowship	Dr. De Paola	07/01/04	06/30/06	34,500
European Molecular Biology Organization (EMBO) Long-term Fellowship	Dr. Da Silva	01/15/05	01/14/06	35,000 *
Fraxa Research Foundation	Dr. Bureau	01/01/05	12/31/05	40,000 *
Francis Goelet Fellowship in Biomathematics	Dr. Wigler	10/01/05	09/30/08	70,672 *
Goldring Fellowship	Dr. Stillman	09/01/00	08/31/06	75,000 *
Hereditary Disease Foundation	Dr. Iijima	05/01/05	04/30/06	58,000 *
Klingenstein Foundation	Dr. Chklovskii	07/01/04	06/30/07	74,376
Leukemia Research Foundation	Dr. Ceparo	07/01/05	06/30/07	30,000 *
The Leukemia & Lymphoma Society	Dr. Buckley	11/01/03	10/31/06	40,000
The Leukemia & Lymphoma Society	Dr. Gangadharan	07/01/05	06/30/08	55,000 *
The Leukemia & Lymphoma Society	Dr. Liu	07/01/05	06/30/08	55,000 *
The Leukemia & Lymphoma Society	Dr. Wendel	07/01/05	06/30/08	55,000 *
The Leukemia & Lymphoma Society	Dr. Tansey	07/01/01	06/30/06	100,000
Life Sciences Research Foundation/ DOE-Energy Biosciences Research Fellowship	Dr. Goto	06/01/04	08/31/05	9,837
Maxfield Foundation	Dr. Lazebnik	12/01/04	11/30/06	5,000
McKnight Endowment	Dr. Huang	07/01/04	06/30/07	75,000
Ministerio de Educacion y Cienia of Spain	Dr. Encinas	05/01/04	04/30/06	32,000
National Parkinson's Foundation	Dr. Sebat	07/01/05	06/30/06	40,000 *
NARSAD	Dr. Ango	07/01/05	06/30/07	30,000 *
NARSAD	Dr. Dicristo	07/01/04	06/30/06	30,000
NARSAD	Dr. Takahashi	07/01/05	06/30/07	30,000 *
NARSAD	Dr. Zhong	07/01/05	06/30/07	30,000 *
Pew Charitable Trust	Dr. Huang	07/01/02	06/30/06	60,000
The Andrew Seligson Memorial Clinical Fellowship for Cancer Research	Dr. Zender	05/01/05	04/30/06	82,300 *
Alfred P. Sloan Foundation	Dr. Brody	09/16/04	09/15/06	24,888
Lauri Strauss Leukemia Foundation	Dr. Ceparo	04/01/05	03/31/06	15,000 *
Swartz Foundation	Dr. Kepecs	01/01/05	12/31/05	54,822 *
Swartz Foundation	Dr. Machens	01/01/05	12/31/05	50,000 *
Swartz Foundation	Dr. Chklovskii	01/01/05	12/31/05	50,000 *
Swartz Foundation	Dr. Chklovskii/Wen	01/01/05	12/31/05	50,000 *
Swartz Foundation	Dr. Uchida	01/01/05	12/31/05	50,000 *
Wellcome Trust	Dr. Khoo	04/01/05	03/31/06	20,000 *
Helen Hay Whitney Foundation	Dr. He	04/01/04	03/31/07	45,000
Helen Hay Whitney Foundation	Dr. Hemann	09/01/03	08/31/06	46,500

Training Support

William Townsend Porter Foundation	Undergraduate Research Program	2005		10,000 *
Steamboat Foundation	Undergraduate Research Program	2005		12,000 *

Course Support

Applied Biosystems	Workshop on Chicken Genomics	2005		1,000 *
ARK-Genomics-Roslyn Institute	Workshop on Chicken Genomics	2005		6,000 *
Cobb-Vantress, Inc.	Workshop on Chicken Genomics	2005		2,000 *

·Includes direct and indirect costs
*New grants awarded in 2005

Grantor	Program	Duration		Amount	
Developmental Dynamics, University of Utah	Workshop on Chicken Genomics	2005		500	*
Essel Foundation	Schizophrenia Workshop	2005		10,000	*
Genesis	Workshop on Chicken Genomics	2005		3,000	*
HHMI	Neurobiology Courses	2005		410,000	*
Medical Research Council	Schizophrenia Workshop	2005		18,182	*
Society For Neuroscience/IBRO Fellowships	Summer courses travel fellowships	2005		17,250	*

Meeting Support

Grantor	Program	Duration		Amount	
Amersham Biosciences	Structural Biology Meeting	2005		1,000	*
BIOGEN IDEC	Target Definition and Vector Design for Molecular Medicine	2005		2,500	*
Bruker Biospin Corp.	Structural Biology Meeting	2005		500	*
Cell Genesys	Target Definition and Vector Design for Molecular Medicine	2005		1,500	*
CGT Corporation	Target Definition and Vector Design for Molecular Medicine	2005		500	*
Fluidigm Corporation	Structural Biology Meeting	2005		1,200	*
GenVec, Inc.	Target Definition and Vector Design for Molecular Medicine	2005		2,000	*
Hampton Research Corp.	Structural Biology Meeting	2005		1,000	*
Health Research, Inc.	Northeast Biodefense Center Meeting	2005		58,370	*
Introgen Therapeutics, Inc.	Target Definition and Vector Design for Molecular Medicine	2005		1,000	*
Louisiana Gene Therapy Research Consortium, Inc.	Target Definition and Vector Design for Molecular Medicine	2005		2,000	*
Merck & Company, Inc.	Structural Biology Meeting	2005		1,500	*
New England BioLabs	Advanced Bacterial Genetics Reunion	2005		1,000	*
Rigaku MSC	Structural Biology Meeting	2005		1,000	*
Vector Logics	Target Definition and Vector Design for Molecular Medicine	2005		2,000	*
Wyeth Holding Corporation	Target Definition and Vector Design for Molecular Medicine	2005		2,000	*

Library Support

Grantor	Program	Duration		Amount	
Josiah Macy, Jr. Foundation		05/01/05	05/31/07	285,247	*
Andrew W. Mellon Fund		05/01/05	09/30/05	23,785	*
New York State		07/01/05	06/30/06	6,588	*
The Rockefeller Foundation and The Rockefeller Archives	*History of Science*	2005		20,000	*

*Includes direct and indirect costs
*New grants awarded in 2005

DOLAN DNA LEARNING CENTER GRANTS

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2005 Funding**
FEDERAL GRANTS			
National Science Foundation	Developing and Disseminating New Laboratories in RNAi and Functional Genomics	06/04–06/06	$ 234,536
National Science Foundation	VCA: Finishing the Rice Genome	09/04–08/06	84,041
National Science Foundation	VCA: Gramene: A Platform for Comparative Genomics	12/04–11/06	113,118
National Institutes of Health	*Inside Cancer*, Multimedia Education Resources for Cancer	01/03–12/05	43,642
Virginia Tech/NIH	Partnership for Research and Education in Plants	09/04–10/05	40,501
U.S.D.A.	Systematic Determination of the Gene Set	02/04–01/06	636
NONFEDERAL GRANTS			
Carolina Biological Supply Company	Research Support	2005	$ 75,000
Clemson University	License, training, and development	2005	50,000
Dana Foundation	*Genes to Cognition (G2C) Online:* A Network-driven Internet Site on Modern Brain Research	10/04–09/06	243,912
Dialog Gentechnik	License, training, and development	2005	12,475
Hewlett Foundation	*Genes to Cognition (G2C) Online*	10/05–10/06	12,922
North Shore–LIJ Health System	DNALC *West* support	2005	50,000
Singapore Ministry of Education	License, training, and development	2005	100,000

The following schools each awarded a grant of $1000 or more for the *Curriculum Study* Program:

School	Amount
Bellmore–Merrick Central High School District	$ 1,250
Commack Union Free School District	1,250
East Meadow Union Free School District	2,500
Elwood Union Free School District	1,250
Great Neck Union Free School District	1,250
Green Vale School	2,500
Half Hollow Hills Central School District	2,500
Harborfields Central School District	1,250
Herricks Union Free School District	1,250
Island Trees Union Free School District	1,250
Jericho Union Free School District	1,250
Kings Park Central School District	2,500
Levittown Union Free School District	1,250
Locust Valley Central School District	1,250
Long Beach City School District	$ 1,250
North Shore Central School District	1,250
Oceanside Union Free School District	2,500
Oyster Bay–East Norwich Central School District	1,250
Plainedge Union Free School District	2,500
Plainview–Old Bethpage Central School District	1,250
Port Washington Union Free School District	2,500
Portledge School	1,250
Ramaz Upper School	2,500
Roslyn Union Free School District	1,250
Sachem Central School District	1,250
Syosset Central School District	2,500
West Hempstead Union Free School District	2,500
Yeshiva University High School for Girls	1,750

The following schools each awarded a grant of $1000 or more for the *Genetics as a Model for Whole Learning* Program:

School	Amount
Baldwin Union Free School District	$ 1,200
Bayshore Union Free School District	2,160
Bellmore Union Free School District	3,100
Bellmore–Merrick Central School District	6,900
Bethpage Union Free School District	1,800
Brandeis School	1,375
East Meadow Union Free School District	2,180
Elwood Union Free School District	3,750
Floral Park–Bellerose Union Free School District	5,150
Friends Academy	2,030
Green Vale School	1,527
Half Hollow Hills Central School District	6,300
Harborfields Central School District	12,360
Herricks Union Free School District	1,920
Huntington Union Free School District	5,827
Lawrence Union Free School District	7,618
Locust Valley Central School District	1,560
Lynbrook Union Free School District	$ 1,360
Mattituck–Cutchogue Union Free School District	1,700
Merrick Union Free School District	1,100
Middle Country Central School District	1,660
North Bellmore Union Free School District	1,070
Old Westbury School of the Holy Child	2,165
Oyster Bay–East Norwich Central School District	2,357
Port Washington Union Free School District	19,505
PS 175, Region 3	15,000
Rockville Centre Union Free School District	4,695
Scarsdale Union Free School District	2,100
South Huntington Union Free School District	7,225
St. Dominic Elementary School	3,630
St. Edward the Confessor School	1,650
Syosset Union Free School District	27,260
Three Village Central School District	2,665

*Includes direct and indirect costs

BANBURY CENTER GRANTS

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2005 Funding*[+]
FEDERAL SUPPORT			
Centers for Disease Control and Prevention (CDC)	From Markers to Models: Integrating Data to Make Sense of Biologic Systems	2005	$ 30,000*
NIH–National Institute of Mental Health (through a grant to University of Illinois)	Translational Approaches to Fragile-X Syndrome: Turning Basic Research Findings into Therapeutic Targets	2005	36,802*
U.S. Department of Defense (through a grant to Children's Tumor Foundation)	Barriers and Solutions in the Use of Mouse Models to Develop Therapeutic Strategies for NF1- and NF2-associated Tumors	2005	20,573*
U.S. Department of Defense (through a grant to Institute of Comparative Genomics)	Pathogenesis and Early Events in Viral Infection	2005	45,878*
U.S. Department of Homeland Security (through a grant to UMDNJ–New Jersey Medical School)	Microbial Forensics 2005: Sample Management	2005	37,500*
NONFEDERAL SUPPORT			
Meeting Support			
Agencourt Bioscience Corporation	A Critical Review of Melanoma: Genomic Approaches with Therapeutic Promise	2005	1,000*
The ALS Association	Stem Cells and Axonal Regeneration: Strategies for the Treatment of ALS	2005	42,174*
CFIDS Association of America	From Markers to Models: Integrating Data to Make Sense of Biologic Systems	2005	5,000*
Chiron Corporation	A Critical Review of Melanoma: Genomic Approaches with Therapeutic Promise	2005	5,000*
The Thomas Hartman Foundation for Parkinson's Research	Parkinson's Disease: Basic Mechanisms and Therapies	2005	28,907*
Ann L. and Herbert J. Siegel Fund of the Jewish Communal Fund	A Critical Review of Melanoma: Genomic Approaches with Therapeutic Promise	2005	25,000*
The Karches Foundation	Chronic Lymphocytic Leukemia	2005	34,301*
Richard Lounsbery Foundation, Inc.	The Biology and Practice of Mammalian Cloning: A Reassessment	2005	54,040*
Melanoma Research Foundation	A Critical Review of Melanoma: Genomic Approaches with Therapeutic Promise	2005	15,000*
PICO Atlantic	A Critical Review of Melanoma: Genomic Approaches with Therapeutic Promise	2005	1,500*
Marie Robertson Memorial Fund	The GABAergic System	2005	20,000
Albert B. Sabin Vaccine Institute, with support of the Bill & Melinda Gates Foundation	Introduction and Sustainable Use of Vaccines in Developing Countries	2005	28,287*
Spinal Muscular Atrophy Foundation	Spinal Muscular Atrophy: Neuronal Rescue and Repair from Laboratory to Clinic	2005	42,448*
The Swartz Foundation	Neurobiology of Decision-making	2005	48,032*
National Society for Epilepsy, with support of UCB Pharma	Epilepsy Genetics and Pharmacogenetics	2005	44,647*
Verto Institute, LLC	Recent Advances in Neuroendocrine Tumor Biology	2005	28,121*

[+]Includes direct and indirect costs
*New grants awarded in 2005

DEVELOPMENT

As you have probably read, 2005 was an exceptional year for Cold Spring Harbor Laboratory. Advances made at the Lab in developing genetic tools that can be applied to the treatment of disease have led to ever-increasing collaborations with clinicians at Memorial Sloan-Kettering Cancer Center and North Shore–Long Island Jewish Health System in New York and as far away as the Karolinska Institute in Sweden. Because the Lab does not have a hospital affiliation, this enables us to work with the best clinicians in the world in a given area of expertise, such as prostate cancer, leukemia, autism, or Parkinson's disease.

This growth has created a need to expand the Laboratory. By adding six new buildings, scientists will have the space and research support to focus more on clinical applications and delve further into tough questions that need complex computer models to decipher. The research and capital required for the Lab to take such a leadership position in designing and applying these genetic tools for patient care are extremely costly. The Laboratory is indebted to our supporters who have invested in our dynamic and enthusiastic scientists as they continue on the frontline in the war against debilitating genetic diseases. The development office has the pleasure of working with smart donors who are optimistic about the future of health care and view the research at this institution as critically important to them and their families.

Lab donors not only take pride in this important work but also know that their gifts are being well spent. For the fifth consecutive year, the philanthropic evaluator *Charity Navigator* has bestowed their highest four-star rating on Cold Spring Harbor Laboratory for its sound financial practices. This distinction ranks the Lab among the most responsible of more than 1.5 million philanthropic organizations that exist in America to date. Less than 12% of the charities rated have received at least two consecutive four-star evaluations, indicating that Cold Spring Harbor Laboratory outperforms most charities in America in its efforts to operate in the most fiscally responsible way possible.

On behalf of the development office, I cannot thank our donors enough for including the Lab in their philanthropic plans and spreading the word about our work. Cold Spring Harbor Laboratory has always been an institution that has taken on great challenges and it will continue this way in 2006 and beyond.

Charles V. Prizzi, Chief Development Officer

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 programs 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 Chief Development Officer, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6865.

CAPITAL AND ENDOWMENT CONTRIBUTIONS

January 1–December 31, 2005

In 2005, Cold Spring Harbor Laboratory received significant support in the form of capital, program, and gifts-in-kind contributions from individuals, foundations, and corporations.

GIFTS $100,000 AND ABOVE

Anonymous*
Mr. and Mrs. Donald Everett Axinn
The Bristol-Myers Squibb Pharmaceutical Research Institute
Edward A. Chernoff*
Landon and Lavinia Clay*
Mr. and Mrs. John P. Cleary*
Shelby Cullom Davis Foundation
The 2001 Frederick & Nancy DeMatteis Family Charitable Trust*
The William Stamps Farish Fund*
Francis Goelet Trust*
Dr. and Mrs. Philip Goelet*
Gladys and Roland Harrimon Foundation
Strauss-Hawkins Trust*
The Lita Annenberg Hazen Foundation
William Randolph Hearst Foundation*
The Koshland Foundation
Laurie J. Landeau, V.M.D.*
The Lehrman Institute
Mr. and Mrs. Robert D. Lindsay and Family
Mrs. George N. Lindsay
Mrs. Edwin S. Marks*
Gillian and Eduardo Mestre*
Mrs. Leslie N. Quick, Jr.
Herbert J. Siegel*
The Simons Foundation
The Starr Foundation
Mr. and Mrs. Henry Wendt, III

GIFTS $5,000–$99,999

Drs. Giovanna Ferro-Luzzi and Bruce Ames
Anonymous
Dr. Sydney Brenner*
Drs. Joan E. Brooks and James I. Garrels
Mrs. and Mrs. Thomas J. Calabrese, Jr.
Cedar Hill Foundation
Robert J. Glaser, M.D.
Dr. Alfred Goldberg
Harrison Family Foundation
Thomas P. Maniatis, Ph.D.
The Rathmann Family Foundation*
Mr. and Mrs. John J. Reese
David Rockefeller
John and Arthur M. Spiro
The Textor Family Foundation
Mr. and Mrs. Richard H. Witmer, Jr.
Karen and Mark Zoller

Total	**$45,286,506.00**

WOMEN'S PARTNERSHIP FOR SCIENCE
June 26, 2005

Committee Cochairs

Kristina Perkin Davison
Kate Seligson Friedman
Blair Husain
Cristina Mariani-May
Deborah Norville
Nancy Tilghman

Supporters ($1,000+)

Robert de Rothschild
Kate Seligson Friedman
Michelle Gerwin-Carlson
Cristina Mariani-May
Gail McDonnell
Brenda Morey
Louise Parent
Debbie Stevenson
Karen Thorson

Women's Partnership cochairs Cristina Mariani-May, Blair Husain, Kate Seligson Friedman, Kristina Perkin Davison, Nancy Tilghman, and Deborah Norville

Total	**$30,367.00**

*New pledges awarded in 2005

BENEFIT FOR THE BRAIN
November 5, 2005

Committee Cochairs
Monique and Doug Morris
Sandy and Howard Tytel

Benefit Underwriter
Mr. and Mrs. Douglas P. Morris

Platinum Benefactors
William S. Robertson–Banbury Fund
Richard A. Rosenbaum–Greenberg Traurig, LLP
Mr. and Mrs. Robert Sillerman–The Tomorrow Foundation
Mr. and Mrs. Howard Tytel

Gold Benefactors
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Mr. and Mrs. Francis J. Ingrassia
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Mr. and Mrs. David L. Luke III
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Dr. Gillian Shepherd Mestre and Mr. Eduardo G. Mestre
Mr. and Mrs. William R. Miller
Pall Corporation
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*New pledges awarded in 2005

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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 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 for 8 representatives of the Corporate Sponsors at our meetings. 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 7,000 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 2005 were:

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Total	**$709,000.00**

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The President's Council was formed 12 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. and M.D.s who are making great strides toward independent and important research. This year, the President's Council raised more than $400,000, establishing a new record for the annual contributions to the Cold Spring Harbor Laboratory Fellows program.

The 2005 meeting focused on a topic to which CSHL President Bruce Stillman has dedicated his own research career: *cancer*. During the 2-day annual meeting, held October 21 and 22, President's Council members had the opportunity to hear presentations from leading scientists, physicians, policy makers, and pharmaceutical companies. Together, these talks and workshops provided a comprehensive understanding of the state of the science in 2005 and a clear vision of what the future holds. The following were members of the 2005 President's Council:

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President's Council members getting a lesson in X-ray crystallography

John Friedman, Don Axinn, and Dr. Peter Scardino

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Association President's Report

It is my distinct pleasure to report that 2005 marked the third year in a row that members of the Association raised more than $1 million for the annual fund. We surpassed our highest goal ever, $1,100,000, and brought in $1,132,475.00 for unrestricted spending for research and programs. The Association annual fund is a crucial source of financing for start-up research projects, purchasing equipment, and providing fellowships for top young researchers. In fact, our supporters are so generous that the Association instituted a new membership level in 2005 for annual gifts of $20,000 and above: "Nobelist."

Once again the Laboratory earned a four-star rating from *Charity Navigator*—a designation awarded by this independent rating service for efficient use of donor funds—joining 1% of the charities rated by the service for earning four stars for five consecutive years in a row.

Not only did we raise more money than ever in 2005, but we made more friends. Thanks to our dedicated board of directors, 40 new members joined the Association in 2005, an important step in our mission to increase awareness of the Laboratory to people beyond our immediate communities.

After 6 years of service, we bid farewell to retiring directors—Alan Kisner, Mary Alice Kolodner, and Larry Remmel—all of whom continue to be active in Laboratory events and outreach. Trudy Calabrese, who retired as the Association President this year after serving for 8 years on the Board, left very large shoes for me to fill. Her enthusiasm, creativity, energy, and particularly her love for the Laboratory were felt by everyone who had the privilege to work with her. Joining the board in 2005 were Joyce Bertoldo, Dick Droesch, Linda Ferrante, and Sandy Tytel.

Our seventh annual Jazz benefit broke all records for attendance and net profits ($110,000 raised). The event, chaired by Joanne and Jay Andrea and Pien and Hans Bosch, drew more than 400 guests who were treated to an extraordinary performance by the multi-Grammy award winning vibraphonist, Gary

2005 CSHLA Directors

Burton. The concert by Burton and guitarist Julian Lage, pianist Vadim Neselovskyi, and bassist Luques Curtis completely raised the bar for entertainment at future Laboratory benefits.

Other successful happenings during 2005 included our annual meeting on February 13 with CSHL scientist Dr. James Hicks. Jim described the powerful new technology developed at the Laboratory, ROMA, or representational oligonucleotide microarray analysis, that analyzes genes and tissue samples for prognosis and diagnosis in breast and other cancers. The technology is evolving rapidly and is now being utilized in the launching of research programs to understand the genetic bases of some neurological disorders, particularly autism.

A wonderful gathering on September 11 for major donors was held on a Sunday afternoon at the home of CSHL friends Nancy and Patrik Edsparr. It was gratifying to hear former Watson School graduate student and current CSHL Fellow Patrick Paddison describe with warmth and good humor his experiences while a student at the Laboratory and his avid devotion to his current project in cancer research.

A number of our members enjoy personal relationships with Laboratory scientists and their families, and our directors hosted a "Partnership Picnic" on Sunday, September 18 to encourage more of these friendships. Director George Cutting, long a champion of promoting friendships with the people who devote their careers to our health, again provided this year fresh Alaskan salmon for the party, caught by him and his family over the summer. We are lucky to have the opportunity to get to know scientists from many different countries, and our partners include these scientists in gatherings of families and friends, a richly rewarding experience.

The Laboratory's annual Symposium's Dorcas Cummings Memorial Lecture and dinner parties occurred on Saturday, June 5. Our keynote speaker, Dr. Charles Sawyers of the University of California at Los Angeles, heads the prostate cancer program there, and he described his contribution to the progress

JAZZ AT THE LAB 2005
April 9, 2005

Jay Andrea, Bruce Stillman, and Pien Bosch at *Jazz at the Lab*

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Emily and Jerry Spiegel (*front*) and Alison and Jim Neisloss at *Jazz at the Lab*

being made in developing molecularly targeted cancer therapies. More than 20 of our members hosted dinner parties for their friends and Laboratory and visiting scientists afterward.

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A visit with Goose Hill Elementary School Students

The annual visit from first graders at the Goose Hill School in Cold Spring Harbor is now in its fourth year. For two mornings in May, the children come classroom by classroom to a specially organized science fair given by enthusiastic young scientists who, with hands-on activities, teach the children about the work they do to understand the brain, cancer, and plants.

New in 2005 was the Laboratory Association's invitation in early December to participate in the Americana at Manhasset's annual *Champions for Charity* shopping benefit. Thanks to chairwomen Pien Bosch, Kate Friedman, Cathy Soref, and Sandy Tytel, many Association members and friends turned out to shop and enjoyed a kick-off cocktail party given by London Jewelers owners Candi and Mark Udell. Results of the shopping spree will be announced in March 2006.

In closing, I could not be more pleased with the achievements of the dedicated members of the Association and the efforts of my fellow Directors. The fund-raising environment in general will not get any easier in 2006. However, we are affiliated with an amazing institution that is making significant advances in basic research at every turn. I believe that we are up for the challenges that lay ahead. I know that I speak for all of the members of the Association who look forward to meeting them head on!

Joseph T. Donohue
President

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Clare Bunce
Angela Cornwell
Jay Datema
Anthony Dellureficio
Charles Egleston
Helena Johnson
Marisa Macari
Gail Sherman
Rhonda Veros
Carlos Viteri
Claudia Zago

Meetings and Courses

David J. Stewart, Executive Director
Mark Beavers
Edward Campodonico
Joseph Carrieri
William Dickerson
Andrew Mendelsohn
Maureen Morrow
Andrea Newell
Kenneth Orff
Valerie Pakaluk
Jonathan Parsons
Lauren Postyn
Richard Schriefer
Mary Smith
Margaret Stellabotte
Andrea Stephenson
Barbara Zane

Public Affairs

Jeffrey J. Picarello, Director
Lisa Becker
Margot Bennett
Miriam Chua
Lynn Hardin
Laura Hyman
Peter Sherwood
John Verity

Special Events

Elizabeth Panagot
Joan Lui

Purchasing

Philip Lembo, Director
Margaret Brock
Carol Brower
Susan DeAngelo
Jeffrey DuPree
Bruce Fahlbusch
Christopher Oravitz
Krystyna Rzonca
Barbara Santos
Leonard Sposato
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