ANNUAL REPORT 2004

COLD SPRING HARBOR LABORATORY

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*November 2004–November 2005

Photo courtesy of The Rockefeller University

Rollin Hotchkiss (1911–2004)

Trustee, Cold Spring Harbor Laboratory, 1954–1966 and 1974–1978

Rollin Hotchkiss was trained as an organic chemist at Yale. He was one of the first to change vocations to the rapidly growing field of microbial genetics. After joining the Rockefeller Institute for Medical Research, Rollin worked with René Dubos on the first antibiotics: gramicidin and tyrocidin. Unfortunately, they were toxic and could only be used topically. Yet it was due to this project that Rollin and I met. In 1945, my teacher from the Bronx High School of Science took me to Rockefeller, because my lab project was to isolate gramicidin. I never dreamt that I would one day be a colleague of his.

When Avery, MacLeod, and McCarty—all at Rockefeller—claimed that, in pneumococcus, DNA acted like genetic material, it caught Rollin's eye. When Avery retired, MacLeod went to NYU, and McCarty went to head the rheumatic fever department in The Rockefeller Hospital, Rollin took up the essential question: How much protein was in the transforming DNA? He found it to be less than .02 of the material—not much protein. Moving on, he took advantage of mutants resistant to sulfanomides. He generalized the phenomenon as they would transform and indicated that DNA was generic genetic material.

With his first wife, Shirley, Rollin had two children: Paul and Cynthia. His second wife, Magda, is a scientist, and they worked together until he retired in 1983.

Rollin was a fun person, always playful. If he had one problem it was that it was difficult to talk with him "mano a mano." He mumbled and talked interminably. One of my students used to take a stopwatch when he went to talk to Rollin. On the other hand, Rollin gave the most clear and witty speeches; I always felt that I was incompetent when we spoke in tandem. Rollin had five graduate students who have all done well. The senior people were P. Model, A. Tomasz, and myself. We will all miss him.

Norton Zinder, Ph.D.
Professor Emeritus
The Rockefeller University

Racker Reading Room, Blackford Hall, 1992

The dedication of Delbruck Laboratory, 1981

Photos courtesy of the Cold Spring Harbor Laboratory Archives

Ralph Landau (1916–2004)

Ralph Landau was born in Philadelphia, Pennsylvania on May 19, 1916. As a high school student there, he read a newspaper article about the new and glamorous field of chemical engineering and immediately decided it was the career for him. As a scholarship student at the University of Pennsylvania, he received a Bachelor of Science in chemical engineering in 1936 and a Ph.D. in chemical engineering from Massachusetts Institute of Technology five years later. Just before completing his doctorate, he married Claire in 1940.

Ralph took a position in 1941 with M.W. Kellogg, one of the first engineering firms that specialized in design and development for the oil refining and chemical industries. Within a few years, he was named head of the chemical department of Kellex Corp., a Kellogg subsidiary, and charged with building a large-scale facility at Oak Ridge, Tennessee, to aid in the efforts of World War II. There, he was responsible for the separation of uranium 235, needed for the atomic bomb, from its predominant isotope, and for designing the equipment to produce fluorine, a highly reactive substance needed to make the uranium hexafluoride used in the gaseous diffusion process.

After the war, Ralph joined Harry Rehnberg, a construction engineer he met at Oak Ridge, and started Scientific Design Company in 1946. The firm eventually became one of the most successful engineering and design firms worldwide, developing and commercializing nearly a dozen processes for producing petrochemicals. Ralph served as Executive Vice President of Scientific Design, then as Chairman and CEO of Halcon International (later Halcon SD Group), and co-founded Oxirane Company with Atlantic Richfield Corp. The company developed many invaluable substances and held several significant patents, but it is most noted for its process for terepthalic acid, the co-product route for the production of propylene oxide, and the development of ethylene glycol by thermal hydration—the chief component of antifreeze and used in the process of making Dacron polyester fiber.

In 1983, Ralph turned his sights to education, serving as a consulting professor of economics at the Stanford Institute for Economic Policy and, the following year, joining Harvard University's Kennedy School of Government as a research fellow.

Ralph's influence spanned the world. The recipient of more than 50 awards and honors, he received both the Fuels and Petrochemicals Division Heritage Award and the John Fritz Medal of the American Institute of Chemical Engineers, in addition to the Chemical Industry Medal, the Perkin Medal, the Winthop-Sears Award for Chemical Entrepreneurship, and the Founders Award of the National Academy of Engineering. In 1985, Ralph received the National Medal of Technology, and in 1997, he was awarded the first Othmer Gold Medal of the Chemical Heritage Foundation. He was a life member of the M.I.T Corporation, a senior trustee of Caltech, a trustee of the University of

Pennsylvania, and former chair of the Princeton University School of Engineering Advisory Council. Ralph was awarded honorary degrees from New York Polytechnic, Clarkson College, Ohio State, and the University of Pennsylvania.

Ralph and I first met in May 1982 upon our jointly receiving honorary degrees from Clarkson College. Apparent immediately was his keen intelligence and likeable self-confidence in making governmental bodies and economic systems operate more effectively. To my surprise, I learned he and his family had a weekend home on Long Island Sound in Asharoken, only 10 miles from the Lab. In July, he and his wife Claire first came to the Lab, with Liz and I soon after spending a delightful evening at their family beach compound. Initially, I feared that he had too many prior obligations to consider being one of our Board of Trustees. But upon being asked, he showed no hesitancy, joining us at our November 1982 meeting.

As a founding member of our Commercial Relations Committee, he helped devise our first policies for commercializing lab discoveries and inventions. He also gave much sound advice for mounting our first Capital Fund Drive (The Second Century Campaign). By his being on the Caltech Board, he was a friend of Arnold Beckman and able to play a key role in facilitating the gift from the Beckman Foundation that made possible the construction of our Neuroscience Center. His greatest long-term legacy to us though was the inclusion of his family in the mission of Cold Spring Harbor Laboratory. His daughter, Laurie Landeau, remains an active member of the Laboratory community, serving both on our Board of Trustees and the Corporate Advisory Board of the Dolan DNA Learning Center.

Ralph and Claire lived life to its fullest, later having a home in San Francisco as well as New York City. From their Russian Hill apartment looking down on San Francisco Bay, Ralph could easily move to and from his faculty apartment and office on the Stanford campus. A high point of every year was the anniversary dinner party held at Lutèce, then New York's premier French restaurant presided over by the legendary chef, Andre Soltner. Liz and I felt most honored to be included on many of these sparkling occasions.

The last several years of Ralph's life were marked by medical complications that restricted his past ability to joyously zip through life. Happily, his brain never slowed down, and he retained the capacity to reflect intelligently on the world's problems until his life ended on April 5, 2004.

Ralph's friendship to many leading academic institutions, his impact on the world of chemical engineering, and his strong values of scholarship, philanthropy, and integrity positively changed the lives of many in the United States and abroad. We will cherish his memory for many years to come.

James D. Watson

David B. Pall (1914–2004)

David Pall was born in Thunder Bay, Ontario, Canada on April 2, 1914. Growing up on a farm with no central heating or plumbing in rural Saskatchewan, he learned to read by deciphering the comic strips in the daily newspaper. By age five, he began volume one of *A History of the World*, a foreshadow of the quest for knowledge that would direct his entire life. In high school, he was deeply influenced by *Madam Curie*, the biography of the chemist Marie Curie, about her discovery that liquids, particularly water, were often contaminated by infectious bacteria, which could be killed by boiling or reduced in number by filtration.

In 1939, he graduated with a Ph.D. in Physical Chemistry from McGill University as the highest-ranking graduate student in Canada. David moved to New York City and was in dire need of friends and anxious to meet a suitable girl to marry. A friend suggested that he phone Hester Blatt, a young chemist. David telephoned Hester but reached her younger sister, Josephine, instead. Josephine responded positively to his call, and on February 3, 1940, the two were married. During the next 12 years, they had three children, Stephanie, William, and Ellen.

In 1941, with the United States at war, David's laboratory had one of only three electron microscopes in existence worldwide and was requisitioned by the U.S. military to work on the highly confidential "Manhattan Project," to develop the atomic bomb. Working with a group of Ph.D.s at Columbia University, David was able to make several significant contributions to a secret project that separated the metal uranium into its two isomers—U232 and U235—the latter heavier, which was used to make the bomb.

With the end of World War II, David realized that he might be able to use some of the technology he developed to improve aircraft reliability by creating stainless steel filters for oil used in the hydraulic system—a key component for landing gear and ailerons. With $3000 financing provided by his McGill friend, Abraham Appel, he founded in 1946 the Micro Metallic Corporation located in a storefront in Forest Hills. By building a furnace to melt stainless steel powder and developing a new method by which to mold the porous sheets, David developed the first successful, economically viable stainless steel filter for aircraft. He quickly moved to larger space in Brooklyn and began producing his filters for the Air Force's F-106, allowing the then-conceptual plane to finally reach production. Key to David's later success was his 1950 recruitment of his neighbor, accountant Abe Krasnoff, who ran the commercial aspects of his ever-growing company that became the Pall Corporation in 1957 after moving to Glen Cove on Long Island.

In the late 1950s, David's wife, Josephine, was diagnosed as anemic, and her physician arranged for her to have monthly donations of transfused blood. The doctor advised David that after about 12 months, her immune system would reject the transfused blood and she would die—as she did in 1959. The 12-month "limit" was caused by white cells contained in the donated blood, which were recog-

nized as "foreign bodies" in the recipient. David devoted his time to learning about the components and functions of human blood and the use of webs of plastic fiber for filtration, and he quickly began development on a filter to remove white cells from transfused blood.

David organized a group of Pall Corporation volunteers to donate blood, which was then passed through experimental masses of plastic fibers. White blood cell counts were then taken to determine the fibers' efficiency in removing white cells. Using the data so obtained, David designed and constructed filters for use when patients are given donated blood. The filters removed substantially all of the white cells from transfused blood. Use of David's filters rapidly became mandatory for blood donations, launching a substantial increase in Pall Corporation sales and earnings. More important, thousands of lives have been saved from his thoughtful invention.

During his active years with the corporation, David Pall acquired about 180 U.S. patents issued with his name as the inventor. In 1998, he retired from Pall Corporation as chairman, exiting a business with sales in excess of $1 billion. In 1990, he was awarded the prestigious National Medal of Technology by President George H.W. Bush. The medal is the United States' highest honor in technology and is awarded to recognize exceptional contributions to the well being of the nation through technological innovation and commercialization.

In 1960, David married Helen Rosenthal Stream, and her two daughters, Jane and Abigail, became his own. Together they developed a major collection of American and European art that graced their home in Roslyn Estates, Long Island. Although Liz and I first met the Palls in 1972, it was not until 1987 that our lives seriously intersected. A chance July meeting at the North Shore Hospital dedication of the Marks-Boas Research Building gave David the opportunity to seek my help. Helen had a serious blood disorder that might be cured by treatment with GCSF, a newly discovered growth factor being developed by the new California biotech company Amgen. My help was sought to let Helen be the first idiopathic neutropenia patient so treated. Knowing I was soon to be in Los Angeles, I arranged afterward to go to Amgen and ask its president George Rathman to let Helen receive GCSF at Memorial Hospital. He happily consented, and her neutropenia soon disappeared. A very grateful David joined our Board of Trustees that November. A year later, I was most pleased when David asked me to join the Board of the Pall Corporation, of which I still am a member. David and Helen gave generously to the Second Century Campaign, later giving monies that allow us to attach the Pall name to a new upper campus cabin used by the attendees of our meetings and courses.

A most cherished feature of Helen and David's marriage was their biannual visits to St. Moritz in Switzerland's Engedine. There they stayed at the massive Suvretta House, whose opulent blue Peacock Room mirrored the gilded lives of the hotel's immediate uphill Iranian royal family neighbors. Being in Switzerland transformed Helen from a breast cancer survivor into an accomplished langlaufer (cross-country skier). David in turn became an expert skier who went straight downhill through his early 80s. I witnessed them so much at home on snow when my first appearance at the World Economic Forum allowed me afterward to take the 2-hour trip by train that separated St. Moritz from Davos. The following year Liz and I went to Switzerland to be their guests for a weeklong holiday, taking care to bring the formal dress expected of those dining in the Peacock Room. Several more invitations to Davos allowed me again to show my inexpertise on snow with my son Duncan, then working in Moscow, joining us in February 1996. David, sensing Duncan's outfit not up to Suvretta standards, gifted him with a blue blazer appropriate to the night.

Helen's strong health was the first to go, her coming down with Parkinson's disease before being diagnosed with the colon cancer from which she died in 1998. Coping with Helen's final months was a great burden for David, whose then untypical memory lapses soon metamorphosed into the awfulness of the Alzheimer's disease from which he died on September 21, 2004 at the age of 90.

David Pall's more than 70 years of intellectual inquiry and discovery generated an extraordinary wide array of Pall products which have much improved peoples' lives throughout the world. We will long remember a man whose firm heart and keen mind stayed on course.

James D. Watson

PRESIDENT'S REPORT

Throughout its history, research in the biological and biomedical sciences has been driven by the passion, intellect, and vision of individual scientists. Even when collaboration produced a great discovery or advance, the investigators concerned brought their unique perspective to the team effort. A large proportion of the research funds provided by the National Institutes of Health (NIH) supports investigator-initiated research. The extent of this support and the pool of available scientific talent are among the chief reasons that the United States has led international biomedical research for many decades. But there are disturbing signs that this lead is being undermined.

Breakthroughs in science are unpredictable. Little did we know that research on flower pigments in the early 1990s or investigation of gene expression in soil worms in 1998 would make possible one of the most surprising biological discoveries in recent times: the ability of small RNA molecules to control gene expression by interfering with either gene transcription or protein synthesis. Now RNA interference (RNAi) is being studied in many laboratories. Greg Hannon and his Cold Spring Harbor colleagues have made important insights into its biochemistry by identifying many of the key enzymes involved in producing small RNAs in the cells which guide destruction of the mRNA that is translated into protein. Recent collaborative studies with Leemor Joshua-Tor on the structure of one of these enzymes have indicated how RNAi-directed suppression of mRNA works.

Understanding the biochemistry of RNAi has allowed Greg to develop libraries of RNAi-based molecules that can selectively turn off the expression of any human, mouse, or rat gene. These libraries are powerful tools for biomedical research and have attracted the interest of biotech and pharmaceutical companies. A collaboration between teams of Cold Spring Harbor scientists led by Greg Hannon and Scott Lowe has shown that the small RNAi molecules can be expressed in animal cells to control gene expression in specific tissues or produce genetically defined tumors for identifying and validating cancer therapy targets. RNAi technology has made possible a new era of genetic analysis in mammalian cells and has stimulated the formation of biotechnology companies using RNAi to screen for new therapies and even the application of RNAi molecules as drugs. Their success would be yet another example of the importance of basic research to medical and industrial development.

Sometimes, the execution of investigator-initiated ideas requires large numbers of scientists to work in a coordinated manner. The development of radar during World War II by a team of scientists assembled at the Massachusetts Institute of Technology and led by Alfred Loomis, as chronicled in Jennet Conant's book *Tuxedo Park*, is an excellent example. This successful, team-driven science followed the breakthrough British discovery of the resonant cavity magnetron, but the team approach was essential to moving the science from basic discovery to important application. Team science has emerged in biological research, most notably in the Human Genome Project where, first, many scientists reached a consensus that obtaining the complete sequence of human DNA would be good for all of research and, second, international teams of scientists worked collectively from 1990 to 2003 to achieve a goal that has transformed how we do biology.

During the past year, I have been engaged in planning such an approach to the improvement of cancer diagnosis and treatment. Asked by the director of the National Cancer Institute, Andrew von Eschenbach, to advise on the best application of new technologies, a small committee chaired by Lee Hartwell and Eric Lander has produced a report that was presented in draft form to the National Cancer Advisory Board in September 2004 and in final form in February 2005. The committee, and many of its advisors, strongly favors the establishment of a national, team-based effort to identify all of the major cancer-causing genes in the approximately 50 major types of human cancer. The approach taken in this Human Cancer Genome Project is the collection of tumor samples from patients who have undergone treatment and identify in their cancer genome the genetic alterations. Such alterations include point mutations in the DNA-coding and -noncoding regions of genes, or amplifications and deletions of genes or segments of chromosomes. We even envisioned

identifying the epigenetic changes in cancer genomes that might lead to the lack of expression of a particular tumor suppressor gene. All of this information, if collected from enough patients, compared with data from conventional pathology, and correlated with clinical outcomes, could be used for the diagnosis and prognosis of tumors, helping to guide oncologists to the best available current treatment. Such DNA-based diagnosis and prognosis relies on the too few therapeutic approaches now available for treating cancer. Importantly, the identification of genes that are overactive in human tumors may suggest new targets for cancer therapy that could be validated using the new RNAi technology. Within a few years, DNA-based diagnosis of human cancer could be in routine clinical use. But will there be enough research funds to make this happen?

Research is supported by the NIH from discretionary funds voted by Congress. Because of the large current federal budget deficits, discretionary spending is now more limited than in recent years, and the latest NIH budget did not keep pace with inflation. Because of existing commitments to 4-year grants, funding for new work is being progressively reduced. Already only one in six applications to the National Cancer Institute (NCI) will be funded, and this proportion may drop to the historic lows of 10% that were experienced in the late 1980s. If a career in science comes to be seen as worrying about obtaining necessary resources, more than accomplishing goals, fewer talented young Americans will apply to science graduate schools. It is already the case that many foreign graduate students are reluctant to study in the United States at the present time because of restrictions on visas and travel. The effect of these trends on all of biomedical research in this country may be challenging and long-lasting. At a minimum, we will be unable to move forward with sufficient speed on high priorities such as in the use of genome information to diagnose and treat cancer.

Restrictions on funding are coinciding with attacks on some kinds of research, particularly in the high-profile area of embryonic stem cell research. Federal support for human embryonic stem cell research is not possible, prompting individual states to fund independent research initiatives. However, specialists in developmental and regenerative biology who live and work in states that lack such support will not easily be able to contribute to this complex area of research, despite their expertise and their potential to improve stem cell science. Even more troublesome are restrictions placed on universities. Because research on human embryonic stem cells cannot be performed in a building where federal research funds are being used, universities and research institutions must build isolated research facilities for embryonic stem cell research, at a cost of many tens of millions of dollars that more logically could support the research itself.

The pressures of limited funding and the political debate about some aspects of biology have led some advocates of biomedical research, often people without training in science, to overstate their case, making claims that research is ripe for major advances in regenerative therapy or hinting that cures are just around the corner. This is almost certainly not true for human embryonic stem cell research. Such blind advocacy is dangerous for the research enterprise, raising false expectations for those afflicted with a disease or disability. But support for this type of research is absolutely necessary because if it is not done, we will never realize the potential that exists.

A widely anticipated report from the National Academy of Sciences is expected in early 2005 to establish guidelines for the advancement of human embryonic stem cell research and I hope that it will be the basis for more rational thinking in this overheated debate. We will only find out if truly valuable treatments will emerge from this or any other promising area of biomedical research if in the future, research is allowed to move forward with adequate funds, we have sufficient ability to attract talent, and there is a reduction in political interference.

Some areas of research, however, are ripe for major inroads in diagnosis and perhaps therapy. Certain types of cancer fall into this category. The success in targeting therapy to molecularly characterized tumors is the wave of the future and now requires coordination and funds. If academia can pull together to approach complex medical problems as a coordinated community, like it did with sequencing the human genome, then we will have made a significant advance in the sociology of science. Such an approach to science, however, should still respect the unique talents of those involved because even in large-scale and coordinated research efforts, individual scientists will generate the ideas to get the job done.

HIGHLIGHTS OF THE YEAR

Research

Genomics and Bioinformatics

By using a powerful and sensitive genome research method they initially developed for cancer gene discovery, Mike Wigler and his colleagues have uncovered what is likely to be one of the most significant sources of normal genetic variation in the human genome. They are now using the same method to begin to study the genetic basis of mental illness and brain disorders including autism, schizophrenia, and Parkinson's disease.

Mike Wigler

The method, called ROMA (for representational oligonucleotide microarray analysis), was developed by Rob Lucito and Mike Wigler. When used in cancer research, ROMA compares the DNA harvested from normal cells and tumor cells. Such "normal-to-tumor" comparisons have already revealed several chromosomal amplifications (excess copies of DNA segments) and deletions (missing DNA segments) associated with breast, ovarian, and pancreatic cancer, as well as leukemia and lymphoma. The identification of these genetic alterations provides the basis for a better understanding of cancer biology and for developing improved diagnostic and therapeutic measures.

In the course of that work, when "normal-to-normal" comparisons of DNA from different individuals were carried out as an experimental control, Mike's lab uncovered several large-scale variations in the human genome that they dubbed copy-number polymorphisms or CNPs. We are all supposed to have two copies of each gene in the vast majority of cells in our body—one from mom and one from dad. But it is now clear that we all have alterations to this Mendelian pattern: Some people have only one copy of a gene due to a deletion, and others have more than two copies due to amplifications of a particular part of a chromosome. In part to aid their cancer gene discovery efforts, Mike, CSHL Senior Fellow Jonathan Sebat, and their colleagues have mapped more than 80 such CNPs in the human genome and found that, on average, the genomes of two individuals differ by about a dozen CNPs. Many more of these CNPs will be discovered.

Mike believes that many CNPs are likely to be associated with inherited susceptibility to neurological and cardiovascular diseases, diabetes, cancer, obesity, or other disorders. His lab has recently set out to use ROMA to search directly for the genetic basis of autism, schizophrenia, and Parkinson's disease. Dr. Scott Powers, once a postdoctoral fellow at Cold Spring Harbor Laboratory, returned as a faculty member this year from industry to ramp up the applications of ROMA to cancer gene discovery.

Molecular and Structural Biology

RNA interference (RNAi) has emerged as a widespread biological regulatory mechanism, as a powerful tool for both basic and applied research, and as a therapeutic strategy of enormous potential. In organisms from fungi and flies to plants and humans, RNAi has an essential multifaceted role in controlling gene expression. Small endogenous RNA molecules produced in the cell, or similar RNAs designed and introduced into cells by scientists, shut off the expression of genes either by blocking transcription of the gene or by blocking the trans-

Leemor Joshua-Tor

lation of the genetic code into protein. One of the best-studied RNAi mechanisms is the quashing of gene expression through the cleavage and destruction of templates for protein synthesis called messenger RNA, a biochemical process worked out in Greg Hannon's laboratory.

Until recently, however, the identity of the molecular scissors that actually cut messenger RNA during RNAi has remained elusive. A collaborative effort led by molecular biologist Greg Hannon and X-ray crystallographer Leemor Joshua-Tor has solved this puzzle by revealing that a protein called Argonaute2 provides the cutting action or "Slicer" activity of RNAi.

Greg and his colleagues focused on sorting out the functions of four distinct yet related mammalian Argonaute proteins (Argonaute1, 2, 3, and 4). With a biochemical approach, they found that only Argonaute2 is part of the multisubunit molecular machine that comprises Slicer activity. To extend these findings, Greg's group showed that messenger RNA cleavage by RNAi is abolished in mouse cells lacking Argonaute2 and that DNA encoding human Argonaute2 could restore Slicer activity in mouse cells lacking Argonaute2. These results were consistent with the idea that Argonaute2 itself provides the Slicer activity of RNAi. However, the possibility that a different protein provides Slicer activity could not be ruled out.

The work of Leemor Joshua-Tor's group clinched the case that Argonaute2 provides the Slicer activity of RNAi. Leemor and her colleagues were studying an Argonaute protein from the archaebacterium, *Pyrococcus furiosus*, by using X-ray crystallography (a method that reveals the three-dimensional structure of molecules at the atomic level). Determining a protein's structure by X-ray crystallography frequently provides valuable, if not decisive, clues about how that protein functions.

When the three-dimensional structure of *P. furiosus* Argonaute emerged from their data and was compared to other proteins of known structure and function, Leemor's group soon noticed that part of *P. furiosus* Argonaute was the spitting image of the "RNase H" family of proteins, whose members were known to cut RNA. With guidance from Leemor, Greg's lab did a final experiment based on the *P. furiosus* Argonaute structure that confirmed Argonaute2 as the protein that provides the Slicer activity of RNAi in mammals. Other information led Leemor to propose a model that explains precisely how Argonaute binds and cuts messenger RNA during RNAi.

The discoveries by Greg, Leemor, and their colleagues are a significant advance toward a comprehensive understanding of one of the most intriguing biological phenomena to be uncovered in recent years.

Cell Biology

In 1958, five years after he helped discover the double helix structure of DNA, Francis Crick coined the term "Central Dogma" to characterize the cellular processes whereby DNA is transcribed into RNA and RNA is translated into protein. Since then, researchers have typically explored individual aspects of these processes in isolation by developing separate systems for studying transcription and translation. David Spector and his colleagues have developed the first system for viewing how the Central Dogma unfolds in its entirety, from DNA to RNA to protein, within living cells.

David and postdoctoral fellow Susan Janicki developed a multicomponent, fluorescence microscopy imaging system in which the DNA near an inducible gene is labeled green, the

messenger RNA encoded by the gene is labeled yellow, and the protein encoded by the messenger RNA is labeled blue. The system was then used to capture time-lapse images in live cells as the inducible gene was switched on: First, the DNA architecture in the region of the gene became less compacted. Next, RNA appeared, was spliced in the nucleus, and subsequently exported to the cytoplasm. Finally, the protein appeared.

David Spector

Although scientists know that protein production involves regulated interactions among many molecules that carry out transcription, RNA splicing, translation, and other processes, they have been unable until now to simultaneously track all of the products of these processes as they are produced and move within living cells.

David and his colleagues have used their system to detect specific events that transform the architecture of chromosomes from a transcriptionally silent state to an actively transcribed state. This work has revealed fundamental information about how genes are switched on and off in the context of living cells. The system is being used by many researchers to explore how a variety of dynamic processes involving DNA, RNA, and protein are regulated in normal cells, as well as how those processes or their regulation might be altered in cancer or other diseases.

Neuroscience

The ability to form long-lasting memories shapes who we are and most often enriches (but sometimes impairs) our lives. Understanding the molecular and cellular principles that underlie learning and memory is one of the principal goals of our neuroscience program. This year, faculty members Roberto Malinow and Tony Zador collaborated in a study of a form of associative, "Pavlovian" learning known as fear conditioning.

Roberto Malinow

In humans, fear conditioning involves the association of an otherwise neutral stimulus (e.g., a particular place or sound) with an unpleasant experience. In experimental animals, in which a tone might be paired with a foot shock, a "freezing" response is used to measure fear. In both humans and animals, long after the initial learning period, the neutral stimulus alone elicits fear.

Roberto's group—in collaboration with Hollis Cline, Karel Svoboda, Linda Van Aelst, and others—has previously uncovered several of the molecular "rules" that govern long-term potentiation (LTP), a process whereby synpases become strengthened, that has emerged as a leading candidate mechanism for long-term memory.

Tony Zador

Those studies focused on a brain region called the hippocampus. They revealed that the controlled movement of neurotransmitter receptors called AMPA (α-amino-3-hydroxy-5-methyl-4-isoazole) receptors into synapses is likely to be a key event in the formation of memory. Importantly, these studies also led to the development of a number of powerful tools that Roberto and Tony used in their recent work.

A region of the brain called the amygdala is known to be required for learning and memory formation during fear conditioning. By using a recombinant version of AMPA receptors that specifically tags newly strengthened synapses, Roberto and Tony first tested whether fear conditioning in rats

leads to the strengthening of synapses in the amygdala. They found that as many as one third of the neurons in the amygdala strengthen synapses in response to fear conditioning.

This finding indicates that rather than being restricted to a comparatively small proportion of neurons, long-term memories are widely distributed among a large proportion of neurons. However, on the basis of other evidence (see below), Roberto and Tony do not believe that this wide distribution of memory-associated synaptic changes serves to make memories resistant to being disrupted (e.g., by brain damage or other perturbations). To test whether the strengthening of synapses in the amygdala is required for learning, the researchers used a different recombinant version of AMPA receptors—one that blocks the strengthening of synapses. They found that blocking synapse strengthening in the amygdala during fear conditioning disrupts the learning process that leads to memory formation.

Interestingly, Roberto and Tony also discovered that blocking synapse strengthening in as few as approximately 10–20% of the relevant neurons was sufficient to impair memory formation. This finding contradicts the conventional view that widely distributed memories are tolerant to perturbation and will change the thinking of many neuroscientists in this field.

Short-term or "working" memory is also an important process that enables us to interact in meaningful ways with others and comprehend the world around us on a moment-to-moment basis. A classic, albeit purely practical, example of short-term or "working memory" is our ability to look up a telephone number, remember it just long enough to dial it, and then promptly forget it. However, working memory is believed to be fundamental to many other cognitive processes, including reading, writing, holding a conversation, playing or listening to music, decision-making, and thinking rationally in a general sense.

Carlos Brody

Carlos Brody is exploring how neurons interact with one another to form neural networks that underlie working memory and other rapid and flexible cognitive processes. As part of an ongoing collaboration with Ranulfo Romo (Universidad Nacional Autónoma de México), Carlos' group is developing mathematical models for interpreting data collected by his collaborators, who use animals (macaque monkeys) to perform a simple task that involves working memory. In one version of the task, Romo's animals were trained to compare an initial stimulus (a vibration applied to a fingertip) with a second stimulus applied a few seconds later and to immediately provide a "yes" or "no" answer to the question: Was the first vibration faster than the second?

This behavior requires the animals to load the initial stimulus into their working memory ("loading phase"), hold information about that stimulus in their working memory ("memory phase"), compare that information to the second stimulus, and then make a decision based on the comparison ("decison phase").

At the outset of the study, Carlos and postdoctoral fellow Christian Machens hoped to develop a mathematical model—based on known properties of "spiking" neurons—that would explain how the brain carries out just the memory phase of the behavior. To their surprise, the simple "mutual inhibition" model they developed yielded a neural network architecture that explains not only the memory phase, but also the loading phase and the decision phase of the behavior. The model makes several predictions about the neurological basis of working memory that can be tested to confirm the likelihood that the model is a significant advance toward understanding fundamental properties of brain structure and function.

The human brain is estimated to contain 100 billion neurons (the number one followed by 11 zeros). Because a typical neuron forms approximately 1000 synaptic connections to other neurons, the total number of synapses in the brain is estimated to be 100 trillion (the number one followed by 14 zeros). The thin projections from neurons that form connections

with one another (axons and dendrites) can be thought of as the biological "wiring" of the brain.

Neuroscientists already know that brain neurons can and do form specific rather than random connections with one another to generate the observed wiring diagram of the brain. However, the precise patterns of such nonrandom connections, how the patterns are formed, and how these patterns underlie the brain's extraordinary information processing capacity are important questions that CSHL researchers are addressing in various ways.

Dmitri Chklovskii and his colleagues are using statistical analysis and mathematical modeling—coupled with in vivo, experimental observations—to search for recurrent, nonrandom patterns of local connectivity within the vast thickets of brain wiring diagrams. Finding such patterns would be strong evidence for the presence of functional modules (e.g., local cortical circuits) that process information. This year, Dmitri and his colleagues have potentially uncovered such functional modules by using two complementary approaches.

Dmitri Chklovskii

In one study, they chose the nematode worm *Caenorhabditis elegans* as a relatively simple model system. Previous studies had determined that this organism has 302 neurons and had partially mapped which neurons connect with which. However, these studies did not characterize nonrandom patterns of connectivity in a rigorous way. When Dmitri and his colleagues completed the worm's wiring connectivity map and considered all 13 possible patterns of connectivity that can occur among three neurons (one such "triplet" pattern being "neuron A connects to B, B connects to C, and A connects to C"), they found that three particular patterns, including the one above, stood out as appearing far more frequently in the *C. elegans* nervous system than they would by chance. They also discovered that some triplet patterns were *less* common than predicted by chance. Taking the analysis a step further, Dmitri and his colleagues found that among all 199 possible quadruplet patterns of connectivity that can occur among four neurons, one particular pattern stood out in *C. elegans* as appearing more frequently than it would by chance.

Significantly, Dmitri and his colleagues considered whether the frequent connectivity patterns or "motifs" that they discovered might be accounted for by previously known principles of neurobiology. They found no such explanation for the existence of the motifs, indicating that further analysis of the motifs may reveal important information about nervous system structure and function.

Because it was based purely on anatomical data collected by electron microscopy, Dmitri's *C. elegans* study did not include information about the strengths of connections between neurons. Therefore, to extend his findings into the physiological realm, Dmitri collaborated with researchers at Brandeis University. The Brandeis group had previously collected one of the largest electrophysiological data sets of its kind ever recorded—measurements of the connectivity of some 3000 individual neurons in the rat visual cortex.

Dmitri recognized that the Brandeis data could be used to explore his ideas concerning functional modules in the brain. He and his colleagues detected some of the very same nonrandom patterns of connectivity in the rat brain that they had observed in *C. elegans*. More importantly, they found that most connections formed by neurons in the rat visual cortex are weak and that the stronger connections (~17% of all connections) account for as much as half of the total synaptic strength of a particular network. In part because more strongly connected neurons fire more reproducibly, Dmitri proposes that strong cortical synapses—with particular connectivities—act as a network "scaffold" that is likely to generate reproducible patterns of activity and have an important role in brain function.

Cold Spring Harbor Laboratory Board of Trustees

At our November Board of Trustees meeting, we traditionally honor those who are concluding their service with us. This occasion was notable this year, since we said a special goodbye and thank you to our chairman William R. Miller, who led the Board through two 3-year terms. He will now join us at future Board meetings as our newest honorary trustee.

Following Bill's departure, the Board unanimously elected Eduardo G. Mestre as our new Chairman, Lola Grace as our vice chair, and Edward Travaglianti as our secretary/treasurer. Their vision and experience will help CSHL to expand its research and educational goals, especially in the fields of cancer and neuroscience. Eduardo, a member of the CSHL Board of Trustees since 2001, is currently vice chairman of Evercore Partners, a leading investment and advisory firm, where he is responsible for the firm's corporate advisory practice. Lola, managing director of Sterling Grace Capital Management, previously served as the treasurer and secretary of our Board as well as chair of the finance and audit committees and a member of the Dolan DNA Learning Center committee. Ed, President of Commerce Bank Long Island and a 34-year veteran of commercial banking in the metropolitan New York market, joined the CSHL Board of Trustees in 2003.

Charles Harris

Susan Hockfield

William R. Miller

Charles J. Sherr

Howard Soloman

Susan Hockfield, a scientific trustee since 1998, concluded her term as a scientific trustee of the Laboratory after being named the 16th President of Massachusetts Institute of Technology—the first woman in the institution's history. Although it is sad to see her leave, we are proud of Susan, who first came to know CSHL as a research scientist here in the 1980s. Scientific trustee Charles J. Sherr, M.D., Ph.D., and individual trustees Charles Harris and Howard Solomon also concluded their terms in November.

We welcomed new scientific trustees Laurence Abbott, Ph.D., of the Volen Center at Brandeis University, and Robert E. Wittes, M.D., physician in chief at Memorial Sloan-Kettering Cancer Center. Laurie Landeau, V.M.D., Nancy Marks, and Jerome Swartz, Ph.D., joined the Board as individual trustees this year.

Watson School of Biological Sciences Commencement Convocation

Founded with the mission to bestow the Ph.D. degree in biology in an unprecedented 4 years, the Watson School of Biological Sciences achieved its goal on April 25, 2004 when Amy A. Caudy, Ira Hall, Patrick J. Paddison, Emiliano Rial Verde, Elizabeth E. Thomas, and

Niraj Harish Tolia became the first graduates of the School. This outcome is owed to the generosity of our benefactors and to the dedication of the faculty, administration, and the outstanding students we successfully recruited.

Patrick Paddison, Ira Hall, Elizabeth Thomas, Niraj Tolia, Amy Caudy, and Emiliano Rial Verde

Awards and Honors

CSHL Professor Lincoln Stein was named the 2004 laureate of the Benjamin Franklin Award in Bioinformatics. The Benjamin Franklin Award in Bioinformatics is a humanitarian award presented annually by *Bioinformatics.org* to an individual who has, in his or her practice, promoted free and open access to the methods and materials used in the scientific field of bioinformatics. Recipients are chosen based on nominations and votes by his/her peers—the more than 8000 members of the organization.

Karel Svoboda

CSHL neuroscientist Karel Svoboda was the 2004 recipient of the Society for Neuroscience Young Investigator Award. The prize is awarded each year at the Society's annual meeting to an outstanding neuroscientist who has received an advanced professional degree within the past 10 years. He was also selected by *Popular Science* magazine as one of its "Brilliant Ten" young scientists in the United States for 2004.

Recognized for his "promise of becoming a leader in research in the cure and treatment of cancer, cerebral palsy, and multiple sclerosis," Senthil Muthuswamy was named a Rita Allen Foundation Scholar. The Rita Allen Award is only awarded to a handful of researchers annually—all of whom have been on a tenure track for no more than three years.

The CSHL Press book, *George Beadle, An Uncommon Farmer: The Emergence of Genetics in the 20th Century,* by Paul Berg and Maxine Singer, was selected by the American Library Association for inclusion in its "Outstanding Academic Titles" (OAT) list for 2004. Outstanding Academic Titles are chosen from among more than 7000 books reviewed "for their excellence in scholarship and presentation, the significance of their contribution to the field, and their value as important—often the first—treatment of their subject." Comprising less than 3% of the 23,000 plus titles submitted, Outstanding Academic Titles have been called "the best of the best."

DNA Interactive (DNAi) DVD, produced by the Dolan DNA Learning Center (DNALC) in association with The Red Green and Blue Company (RGB) and Windfall Films, was named "Best Offline Factual" at the British Academy of Film and Television Arts (BAFTA) Interactive Entertainment Awards ceremony—the British version of the Oscars—in February. In its sixth year, the BAFTA Best Offline Factual award is given to "the most imaginative and effective use of offline interactivity to explore the factual world." The *DNAi* DVD was recognized as "an unusually rich and deep experience, stimulating personal exploration of the history, science, issues, and future of the genome from the unique perspective of the people involved in its research."

DNA Interactive DVD

In April, my colleague and friend Tom Kelly of the Memorial Sloan-Kettering Cancer Center and I were awarded the Alfred P. Sloan, Jr. Prize, one of three awards given annually by the

General Motors Cancer Research Foundation (GMCRF). The Sloan Prize recognizes the most outstanding recent contribution in basic science related to cancer research.

The Sabin Vaccine Institute honored CSHL Chancellor James D. Watson, Ph.D., with the Sabin Humanitarian Award at their *Salute to Lifesaving Discoveries* benefit dinner in May. The awards program is a yearly tradition for the Institute and extols the contributions made by scientists, philanthropists, and humanitarians who share in some aspect of the goals of advancing vaccine science for the benefit of humanity.

The United States Rice Genome Consortia, Cooperative State Research, Education, and Extension Service (Tucson, Arizona)—of which Cold Spring Harbor Laboratory is a principal member—were honored with the U.S. Department of Agriculture (USDA)'s Secretary's Award, presented by Agriculture Secretary Ann M. Veneman at the 58th Annual Secretary's Honor Awards Ceremony on June 25. Considered the highest award the USDA can bestow, the members of the United States Rice Genome Consortia were honored in the "Enhancing Economic Opportunities for Agricultural Producers" category for leading the United States partnership in the multinational achievement to decode the rice genome to advance knowledge, improve nutrition, and alleviate world hunger. W. Richard McCombie, Melissa Kramer, Lance Palmer, Robert Martienssen, Maureen Bell, Sujit Dike, Lidia Nascimento, Andrew O'Shaughnessy, and Lori Spiegel are among the members of the CSHL staff involved in this project.

Development

Capital and Program Contributions

Private funding is essential to our research programs, enabling successful and innovative projects not yet eligible for public funding. For this reason, we are especially grateful to those supporters who made major gifts in 2004 to our cancer and neuroscience research programs. We gratefully acknowledge donors of $100,000 or more to our cancer program: the DeMatteis Family Foundation, a first-time grant for colon cancer research; The Miracle Foundation; The Breast Cancer Research Foundation; and one anonymous donor.

Our neuroscience program was also generously supported, and we acknowledge donors to that program, including Jo-Ellen and Ira Hazen, The Seraph Foundation, The Dart Foundation, The G. Harold and Leila Y. Mathers Charitable Foundation, and the St. Giles Foundation. We also received a special gift this year from Trustee Jerome Swartz, and his Swartz Foundation, which provided more than $215,000 for the establishment of the The Swartz Center for Computational Neuroscience and additional research support in brain structure and neuroscience research. The Swartz Center has become an integral part of our strong neurobiology program, supporting both research and neuroscience programs. The Thomas Hartman Foundation for Parkinson's Research also pledged $4.4 million over the next 5 years to support Parkinson's research at CSHL and to establish The Thomas Hartman Parkinson's Research Laboratory. We also received significant and continued support from The Simons Foundation, to fund autism research at CSHL.

Robertson Research Fund

The Robertson Research Fund has been the primary in-house support for our scientists for more than three decades. During 2004, Robertson Funds supported research in the labs of Josh Dubnau, Masaaki Hamaguchi, Leemor Joshua-Tor, Alexei Koulakov, Adrian Krainer, Yuri

Lazebnik, Wolfgang Lukowitz, Bud Mishra, Partha Mitra, Scott Powers, Cordula Schulz, and Michael Wigler.

Watson School of Biological Sciences

Now in its second phase of funding and led by Robert D. Lindsay, the Watson School has received additional support in 2004 for the Dean's Chair, fellowships, and lectureships, enabling 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 Mr. and Mrs. Robert D. Lindsay and Family, Curt Engelhorn, and The Seraph Foundation, as well as ongoing support received from Bristol-Myers Squibb Company, Mr. and Mrs. Alan E. Goldberg, the Florence Gould Foundation, and the Lita Annenberg Hazen Foundation.

The Dolan DNA Learning Center

Thanks to a very generous gift from The Dana Foundation, the Dolan DNALC has embarked on creating *Genes to Cognition (G2C) Online: A Network-driven Internet Site on Modern Brain Research,* an Internet portal exploring the genes of cognition and learning. In addition, the Dolan DNALC received significant support from the Pfizer Foundation to continue its Pfizer Leadership Institute in Human and Genomic Biology.

Carnegie Building

We are continuing plans to renovate the existing Carnegie Library to enhance its ability to serve the Laboratory community. This project received significant support this year from alumni Philip A. Sharp, Ph.D.; Drs. Joan Brooks and James Garrels; and Thomas P. Maniatis, Ph.D.; and from The Koshland Foundation and The Lehrman Institute.

Capital Campaign

As CSHL prepares to embark on a capital campaign to raise funding for new research space, we are very grateful to significant gifts received to support the buildings intended for the southwest corner of our campus. In 2004, we received pledges from Mrs. Leslie C. Quick, Jr., for a cancer research facility named for her husband, and a pledge from the Wendt Family Charitable Foundation of Community Foundation Sonoma County for a neuroscience research facility. Mrs. William L. Matheson made a significant gift in 2003 for this project, to name a facility for her late husband. CSHL also received significant support for these projects from Gillian and Eduardo Mestre and Mrs. George N. Lindsay. We will continue to plan this campaign throughout 2005.

Additional Support

The Laboratory was fortunate to receive support for many ongoing projects in 2004. The Joseph G. Goldring Foundation made a significant gift to support research, and the Louis Morin Charitable Trust made a significant contribution to support the work of Drs. Leemor Joshua-Tor, Josh Dubnau, and David Spector. We received support from the Estates of Elisabeth S. Livingston, Florence Strelkowski, and Adele C. Diaz to complete our housing

project at Uplands Farm, and Dr. and Mrs. Walter C. Meier, through the Banbury Fund, made a significant gift to restore and renovate Robertson House on our Banbury campus. Mr. and Mrs. Charles E. Harris II made a gift this year to support capital projects, and the Roy J. Zuckerberg Family Foundation provided support for consultants in fund-raising and development. We are very grateful to these close supporters for their continued generosity.

Breast Cancer Groups

A crucial component to our breast cancer research program is the support we receive from local grassroots breast cancer groups who provide direct research support for our program, in addition to a myriad of patient care and educational services to thousands of constituents. This year, we were fortunate to receive support from Long Islanders Against Breast Cancer (L.I.A.B.C.); 1 in 9: The Long Island Breast Cancer Action Coalition; Breast Cancer H.E.L.P., Inc.; the New York State Grand Lodge Order Sons of Italy; F.A.C.T. (Find a Cure Today); The Elisabeth McFarland Fund; The Long Island 2 Day Walk to Fight Breast Cancer; The Judi Shesh Memorial Foundation; the Long Beach Breast Cancer Coalition; and The WALK for Women Breast Cancer Fund. We also gratefully acknowledge continued support from The Breast Cancer Research Foundation. The generous support we receive from these groups, year after year, is truly propelling our breast cancer research.

Benefit for the Brain

John Sebastian and the J-Band played to a packed Grace Auditorium on October 30, 2004 at the first "Benefit for the Brain." The event realized a net profit of more than $225,000 for

John Sebastian

Kay Jamison signing a copy of *Exuberance* for Joan Spiro.

CSHL's Alzheimer's and Parkinson's research, thanks to the efforts of William S. Robertson and the Banbury Fund, who underwrote the event, and event co-chairs Edward Travaglianti and Kathy DiMaio and their committee. The event honored Monsignor Thomas J. Hartman, founder of The Thomas Hartman Foundation for Parkinson's Research.

Exuberance: The Passion for Life

On December 5, Dr. Kay Redfield Jamison, best-selling author and internationally renowned authority on mood disorders, discussed the feeling of exuberance and how it fuels our most important creative and scientific achievements at the Dolan DNALC. Sponsored by Arthur and Joan Spiro, this event benefited the educational opportunities hosted annually at the DNALC.

Library and Archives

The Library and Archives enjoyed a very exciting year in 2004. A major goal of the Library is to provide scientists with digital access to all publications vital to their research. In response to the expanding scope of science research performed at the Laboratory, the Library subscribed to many new journal titles this year. In addition, the Library purchased an electronic book collection composed of 134 e-books. In 2004, an SFX system was installed that electronically interconnects the Library's resources so that patrons can navigate the entire Library collection through one interface. Within the past year, the Interlibrary Loan office implemented *Ariel*, an advanced electronic delivery service that allows the Library to receive requested articles as PDFs via e-mail.

The Library's Advisory Committee, composed of professors, postdocs, and Watson School students, convened this year to discuss library services and resources. In 2004, the Library collaborated on particular projects with other medical and academic libraries at The Rockefeller University, Harvard University, and the Marine Biological Laboratory at Woods Hole. The Library, represented by director Mila Pollock, was also invited to the Annual Nature Publishing Group International Library Committee to take part in discussions of interest to today's library market.

In 2004, the Archives were fortunate to acquire the personal collection of Elof Carlson, a prominent geneticist and professor at Stony Brook University. The *Oral History Office* has also continued to expand, now comprising more than 100 scientific interviews, half of which are already accessible online. In 2004, the Sloan Foundation formally recognized the digital archives project, the *Memory Board*, an online forum in which the lab community documents the history of the Laboratory by contributing first-hand accounts, reminiscences, original materials, photos, and video clips. The Sloan Foundation will support the redesign of the Memory Board and advocate its use as a model that other institutions could adapt for documenting their own history. Conceived by librarian and archivist Mila Pollock, the Memory Board has attracted much attention and many interesting anecdotes. The exhibition *Building Blocks of CSHL* was created this year and will be on display at Blackford Hall for two years and online in digital format. This project explores the history of the buildings at CSHL (and the life of its community) through documents, photos, and personal memories. This year, the Library's exhibit *Honest Jim: Watson the Writer* moved to the Charité Universitätsmedizin in Berlin, Germany and will be exhibited in Moscow, Russia next year.

Building Projects

The Laboratory continues to improve and expand its facilities and, during 2004, undertook several construction and renovation projects. Several projects were completed in the Demerec building, including alteration and renovation of laboratory spaces and the construction of a new tissue culture laboratory. Grace Auditorium received attention as well, with a complete remodeling of the bookstore to accommodate *DNA Stuff*—the Laboratory's bookstore and nonprofit retail operation. The Laboratory also continued its project to replace the HVAC systems in Grace and Harris and to update the emergency power system that supplies those buildings.

A major renovation of the James building was begun in 2004, updating laboratory, support, and office spaces to meet the increased demands on the building's facilities the Laboratory has experienced in recent years. Work was begun on the Banbury pool, which had been leaking badly for years. The entire pool has been reconstructed, and final work is to be completed by the spring of 2005.

The Laboratory has continued to improve its student and scientist housing program. The housing project at Uplands Farm is nearing completion and will provide much-needed housing close to the main campus. The last of the apartments in the Hooper building was renovated, and the Weghorn House, which lies at the northern tip of the campus, was purchased and will be renovated in 2005.

A major milestone in 2004 was the approval of the Laboratory's updated Master Plan and its Upper Campus project by the Village of Laurel Hollow. This approval came at the end of a 2-year process and has paved the way for the Laboratory to break ground on the largest construction project it has ever undertaken in Spring 2005.

100 Years of Genetics at Cold Spring Harbor

In 1904, the Carnegie Institution of Washington founded a Station for Experimental Evolution at Cold Spring Harbor, launching a century of genetics that placed CSHL at the forefront of biomedical research. The occasion was marked by a very special 2004 Cultural Series filled with engaging lectures and inspiring music and art.

Public Lectures

April 19 Mark Hallett, Vice President of the American Academy of Neurology: *How Your Brain Recovers from a Stroke.*

May 11 Vincent Li, Director of the Angiogenesis Foundation: *Prevention and Reversal of Skin Cancer and Other Skin Diseases.*

May 18 Carter Burwell, award-winning composer: *From Cold Spring Harbor to the Coen Brothers: A Composer's Journey.*

May 24 Andrew Solomon, award-winning author of *The Noonday Demon: An Atlas of Depression*: *Depression Too, Is a Thing with Feathers.*

Sept. 28 Zach Mainen, CSHL Associate Professor, and Sharron McCarthy, Director of the Society of Wine Educators: *Demystifying the Sommelier: The Art and Science of Wine Tasting.*

Sylvia Nasar

Antoine Tamestit

October 5 Sylvia Nasar, author of *A Beautiful Mind: Genius, Madness, Recovery*.

December 7 Richard Stone, Britain's youngest royal portrait artist: *Painting England's Queen and DNA's Dean*.

Concerts

April 24 The Molinaro-Levy Project, piano and harmonica
May 1 Yunjie Chen, piano
May 8 Mikhail Simonyan and Alexei Podkorytov, violin and piano
May 22 Hsing-ay Hsu, piano
September 11 Dmitri Berlinksky and Elena Baksht, violin and piano
October 2 Vassilis Varvaresos, piano
October 9 Alexandre Bouzlov, cello
October 16 Antoine Tamestit, viola

Exhibits

From July 9 to August 1, 2003–2004 Artist-in-Residence Eduardo De Soignie displayed his work, *Paintings From Another Domain*, in Bush Lecture Hall. Inspired by his Cuban roots, De Soignie's work is influenced by artists such as Jose Bedia and Tomas Esson.

Events

Gavin Borden Visiting Fellows

The 10th annual Gavin Borden Fellow Lecture, created by Jim Watson in memory of Gavin Borden, publisher of *Molecular Biology of the Cell*, was held on March 17 in Grace

Richard Losick and Bruce Stillman at the Gavin Borden Lecture

Auditorium. Dr. Richard Losick, the Maria Moors Cabot Professor of Biology at Harvard University, spoke on "Cell fate, polarity, and cannibalism in bacteria." Dr. Losick is internationally acclaimed for his research on microbial development and is developing computer-based animations and video for teaching introductory molecular biology.

Symposium

The 69th Annual Cold Spring Harbor Laboratory Symposium, "Epigenetics," was oversubscribed again this year with nearly 500 scientists from around the world in attendance. The Dorcas Cummings Lecture, in memory of long-time Laboratory friend and former Director of the Long Island Biological Association Dorcas Cummings, was given by David Haig of Harvard University on the subject of "The Divided Self—Brains, Brawns, and the Superego." This endowed lecture is traditionally open to friends and neighbors of the Laboratory and is followed by dinners generously hosted in the homes of our neighbors for our visiting scientists and faculty and staff.

Other Lectures

Winship Herr ("Why Our Cells Multiply") and Nick Tonks ("Plague, Pox, and Phosphatases") 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.

Huntington Hospital continued to host their fall/spring lecture series on cardiovascular health and related diseases in Grace Auditorium.

Laboratory Employees

New Staff

Scott Powers returned to CSHL in October as Associate Professor and Director of the Human Cancer Genome Center in the Cancer Genome Research Center. Scott came from Tularik Genomics Division, which was initially a company spun out from the Laboratory to use RDA (representational difference analysis) technology to identify cancer genes. Scott received his graduate degree from Columbia University and performed seminal work as a postdoc with Mike Wigler on the study of *RAS* genes in yeast.

David Wu joined Scott as a Research Investigator at the Human Cancer Genome Center. David received his graduate degree from Berkeley and as a postdoc with Aziz Sancar developed the first biochemical system for mammalian DNA repair with purified proteins.

Promotions

Dr. Lilian Clark Gann became Dean of the Watson School of Biological Sciences on July 1. Lilian joined the Laboratory in March 1999 as Assistant Dean of the Watson School and was promoted to Associate Dean in January 2002. She received her Ph.D. from the University of St. Andrews, Scotland in 1988, for her studies on DNA–protein interactions in transcriptional control elements of DNA tumor viruses, and her M.B.A. from the University of Westminster, London in 1996. Since her arrival at CSHL, Lilian had played a crucial role in the development of the School's innovative Ph.D. program, while also enhancing the educational and training environment of CSHL for students and postdoctoral fellows in general. Her unique background in science, education, and business made her an obvious choice to develop and oversee the programs of the Watson School. She was joined by Dr. Bill Tansey who was appointed as director of graduate studies in the Watson School of Biological Sciences and Lita Annenberg Hazen Professor of Biological Sciences on the same day.

A number of other faculty members were promoted in 2004, including Dmitri Chklovskii to Associate Professor, Zachary Mainen to Associate Professor, Ravi Sachidanandam to Senior Computer Scientist, Lincoln Stein to Professor, Karel Svoboda to Professor, Yi Zhong to Professor, and Anthony Zador to Associate Professor. Ira Hall and Patrick Paddison were both named CSH Fellows.

Departures

Dr. Winship Herr stepped down as Dean of the Watson School of Biological Sciences effective July 1 to concentrate his efforts on his research. Beginning in 1995, Winship spearheaded the effort that resulted, in September 1998, in the Laboratory's accreditation as a Ph.D. degree-granting institution by the Board of Regents of the University of the State of New York, on behalf of the State Education Department. This enabled the establishment of the Watson School of Biological Sciences. Soon thereafter, Winship became the Founding dean of the Watson School. During the last 5 years, we have seen the School grow and become one of the most innovative programs in the country, attracting outstanding students. Winship has nurtured and shaped the Watson School in its first formative years, culminating in our first commencement convocation this spring. In the coming year, he and Nouria Hernandez will move to Nouria's native Switzerland to become professors at a new institute in Lausanne. I thank Winship for his remarkable effort in establishing a graduate school at Cold Spring Harbor and for his sound advice to me. The establishment of a graduate school

has transformed Cold Spring Harbor Laboratory and will have a long-lasting effect on the intellectual environment here.

Neilay Dedhia, Research Investigator; David Helfman, Professor; Terence Strick, CSH Fellow; and Jerry Yin, Associate Professor, all departed the Laboratory in 2004. David spearheaded the understanding of cancer progression through his research on the cell biology of cell architecture and I wish him the best as he continues his research at the University of Miami Cancer Center.

Long-term Service

The following employees celebrated milestone anniversaries in 2004:

30 years	Lane Smith
25 years	Maureen Berejka, Judith Cuddihy, Katya Davey, James Hope, John Meyer, James Parsons, Susan Schultz, Bruce Stillman
20 years	Carmelita Bautista, Dessie Carter, Robert Gensel, Mary Ellen Goldstein, Daniel Miller, Robert Pace, Steven Tang
15 years	Leslie Allen, James Bense, Sharon Bense, Charlene De Poto, Janice Douglas, Jan Eisenman, Helena Johnson, Robert Martienssen, Jacqueline Matura, Alison McDermott, Eleanor Sidorenko, Halina Swidzinski, Ryszard Swidzinski, Spencer Teplin

1st row, left to right: Eleanor Sidorenko, James Bense, Sharon Bense, Charlene De Poto, Susan Schultz, Carmelita Bautista, Jan Eisenman, Katya Davey
2nd row, left to right: Rob Gensel, James Parsons, Mary Ellen Goldstein, Alison McDermott, Maureen Berejka, Daniel Miller
3rd row, left to right: John Meyer, James Watson, Spencer Teplin, Helena Johnson, Dessie Carter
4th row, left to right: Bruce Stillman, James Hope, Steve Tang

CSHL team at the Long Island Walk to D'Feet ALS.

Community Outreach

In May, Laboratory employees Sandy Neuwald, Lisa Manche, Carla Margulies, Barbara Misk, Kathryn Borowski, Barbara Purcell, and Theresa Saia served dinner at the Ronald McDonald House in nearby New Hyde Park, which provides temporary housing to families of seriously ill children at the Schneider Children's Hospital. In June, Shuly Avraham, Jason Evans, Kim Gronachan, Jo Leonardo, Jianli Li, Erin Maroney, Liya Ren, Theresa Saia, and Doreen Ware planted six flats of flowers at Haven House, a homeless shelter in Huntington Station, purchased with money donated by CSHL employees. A CSHL Team also participated in the *Long Island Walk to D'Feet ALS* (Lou Gehrig's disease) for the The ALS Association Greater New York Chapter at Eisenhower Park in East Meadow on September 26.

Cold Spring Harbor Laboratory is a vibrant and dynamic institution, and like all such institutions involved in the increasingly expensive research in the genomic age, we are even more dependent on support from individuals and foundations to enable us to remain one of the leading research centers in the world. As we move forward, we need the type of support that can be used to ensure that our scientists have the facilities and resources to achieve their goals. Increasingly at Cold Spring Harbor, our research focuses on how to use all the accumulated knowledge to develop new ideas for diagnosing and treating human disease. I thank all those who have supported our efforts to date, helping us to make Cold Spring Harbor Laboratory a truly unique education and research center.

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

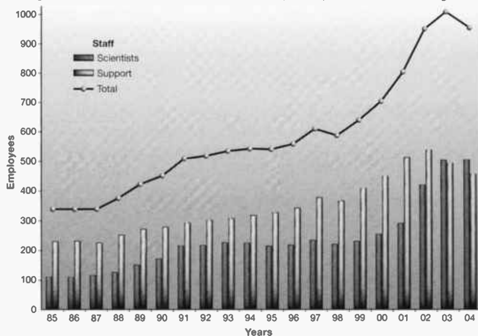
CHIEF OPERATING OFFICER'S REPORT

There are many positives to report about the Laboratory's performance in 2004. The research and academic programs continued to flourish. The endowment funds enjoyed another outstanding year of investment returns and appreciation. Operating results exceeded forecasts and the budget was balanced after depreciation expense. The latter, however, was accomplished only with rigorous management of administrative expenses and some extraordinary factors.

Drs. Watson and Stillman have been heard to say, over the last year, that the research program at the Laboratory is more exciting now than it has ever been in their experience. Having now worked at Cold Spring Harbor Laboratory for 38 and 26 years, respectively, their observations carry considerable weight. Powerful technologies and techniques, developed here at Cold Spring Harbor, allow for rapid identification of genes associated with various cancers as well as psychiatric diseases such as autism, depression, and schizophrenia. The promise of the "DNA Biopsy" for tailoring therapy to an individual's specific genetic alteration is extraordinary and suddenly attainable.

Our academic programs remain strong and continue to expand. Cold Spring Harbor Laboratory Meetings and Courses are the most sought after in the world of biology; they attracted a record number of attendees in 2004. The Cold Spring Harbor Laboratory Press, which continues to publish high-quality scientific books and journals, had an excellent year as well. The Dolan DNA Learning Center is fulfilling its mission of providing unique educational experiences for middle and high school students via hands-on laboratory and Internet instruction. The Banbury Center continues to attract scholars from around the world to its specialized scientific conferences. The innovative Watson School of Biological Sciences celebrated its first commencement in 2004, awarding the degree of Ph.D. to six outstanding students, all of whom completed their degrees in the requisite four years. Honorary degrees were awarded to former Laboratory Trustees Joan Steitz (Professor of Molecular Biophysics and Biochemistry at Yale), Shirley Tilghman (President of Princeton), and, most fittingly, to James D. Watson.

Forecasting the Laboratory's operating revenue is a challenging task due to the varied nature of the revenue streams and shifts in timing that are difficult to anticipate. Consequently, a relatively conservative approach is taken in the budgeting process as we project income from grants, meetings, book sales and advertising, unrestricted fund-raising, and technology licensing activities. We anticipated another difficult year in 2004 and forecasted a deficit from operations of $775,000 after $5.7 million in depreciation expense. Fortunately, results were better than expected, and a balanced budget was achieved for the year. Operating and program income rose to $95.4 million, an 8% increase over 2003. Indirect cost revenue on grants was ahead of forecast and the Cold Spring Harbor Laboratory Press exceeded expectations on stronger book and journal sales combined with printing cost reductions. It should be pointed out, however, that one accounting change involving the allocation and timing of certain expense items had the extraordinary effect of improving the reported bottom line results for the year by approximately $200,000.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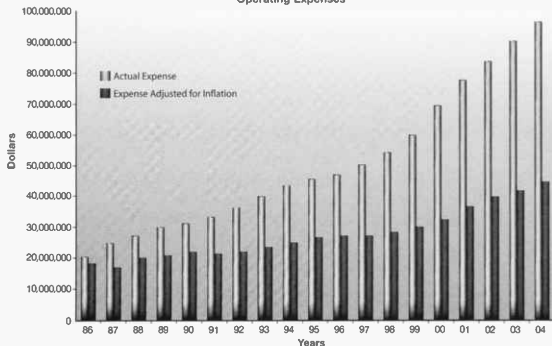
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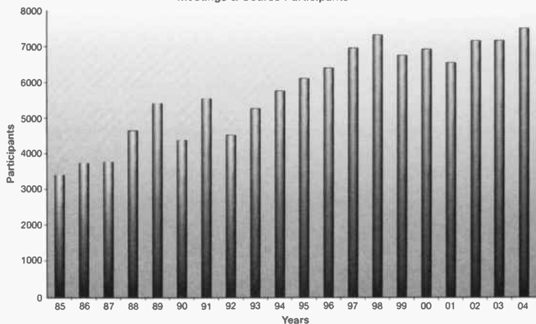
Meetings & Course Participants
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Years

It is certainly satisfying to be able to report a balanced budget, particularly after experiencing substantial operating deficits in the two prior years. This, however, was not easily achieved and required aggressive management of expenses, including caps and deferrals of administrative salary increases. Although such steps were necessary and prudent, they present very real difficulties for hundreds of loyal employees whose lives are very much affected by rising fuel prices, property taxes, and other cost of living pressures that outpace their compensation increases.

Investment returns on our endowment funds were exceptionally strong for the second consecutive year. The total managed portfolio returned 12.1% for the calendar year versus our benchmark index of 8.2%. Our total equity portfolio was up 15.7%, considerably ahead of the 10.9% appreciation in the Standard & Poor's. The fixed-income portfolio also outperformed the indexes, returning 5.1% for the year. At year-end, the market value of the endowment funds totaled $235 million after following our long-held policy of spending no more than 4% annually. The Investment Committee has functioned well and has worked throughout the year with our advisor, Merrill Lynch Consulting Group, to review and refine the asset allocation model and strategy. To diversify and lessen correlation in the equity portfolio, three new managers were added—two in international equities and one in the alternative asset/hedge fund category. We express our gratitude to Lola Grace for her years of hard work as Secretary/Treasurer of the Board and Chair of this committee. At year-end, she passed the baton to Ed Travaglianti, who has assumed these important roles with equal wisdom and enthusiasm.

One of the reasons for the ongoing success and vitality of the Laboratory is the wonderful support it receives from the local community. The Cold Spring Harbor Laboratory Association has a long history of volunteer outreach and involvement with the Lab. Its directors work hard to communicate the Laboratory's mission, welcome scientists into the local community, organize events, and raise unrestricted funds to support critical research. Trudy Calabrese has completed a particularly successful term as president of this important organization. Under her inspired leadership, we enjoyed another year of wonderful concerts, dinners, and lectures and, at the same time, surpassed the $1 million threshold with our Annual Fund for the second consecutive year. We thank Trudy for her dedication to the Laboratory, and we welcome her successor, Joe Donohue.

A great deal of progress was made over the course of the year toward the Laboratory's plans to construct a cluster of new research buildings on the main campus. Chief Facilities Officer Art Brings worked very effectively with the diligent, but cooperative, Village of Laurel Hollow Board of Trustees to obtain approval for the project. It was a complex process involving the preparation of an amazingly detailed environmental impact statement that required review by numerous local and state agencies. By year-end, the necessary approvals were in place and a building permit should be forthcoming early in 2005. Ground breaking on the site is planned for the spring.

Clearly, critical expansion plans cannot be realized without major private financial support. Last year, we acknowledged the generous pledge from long-time friend Mardi Matheson for funds to construct one of the cancer research buildings. Her generosity, along with a $20 million pledge from the State of New York, allowed us to confidently move forward with the project. In 2004, we received two additional major gifts toward the initiative, also from long-time friends of Cold Spring Harbor. Jean Quick, wife of late CSHL Trustee Leslie Quick, pledged $10 million for the second cancer research building. Ongoing support and involvement from families like the Quicks and the Mathesons is immensely gratifying. Mrs. Quick's gift was fol-

lowed, at the end of the year, by another $10 million pledge from former CSHL Trustee and pharmaceutical industry executive Henry Wendt and his wife Holly. In addition, Trustees Bob Lindsay and Howard Solomon made seven-figure contributions to the effort. We express our tremendous gratitude to these individuals for their generosity and commitment to the institution and its future.

The Laboratory could not function effectively without a strong Board of Trustees. Five valued members of this body—Charles Harris; Susan Hockfield, Ph.D.; Charles J. Sherr, M.D., Ph.D.; Howard Solomon; and William Miller—completed their terms at year-end. We are truly grateful for their years of service, advice, and dedication. We express particular gratitude to Bill Miller who served on the board for 15 years, the last six as chairman. His devotion of time, energy, wisdom, and resources to Cold Spring Harbor Laboratory has been of immeasurable value to the institution. We are pleased that he was immediately elected as an Honorary Trustee and welcome his continued involvement. We also welcome and look forward to working with newly elected Trustees Laurence Abbott, Ph.D.; Robert Wittes, Ph.D.; Laurie Landau, V.M.D.; Nancy Marks; and Jerome Swartz, Ph.D. It is comforting to see smooth and seamless leadership transition on the board. We thank Eduardo Mestre for assuming chairmanship of the Board and look forward to his leadership.

It is exciting to embark on a new stage in the growth of Cold Spring Harbor Laboratory—new directions in promising areas of research, expanding facilities, and a growing student body. There is much to look forward to and much planning to be done, but there are also significant challenges to be faced. Our funding requirements for capital projects, endowment, and research are substantial. We are, for the time being, enjoying relatively robust financial markets and economic conditions that create a positive environment for fund-raising. On the other hand, the federal budget deficit is necessitating a belt-tightening that will have a major impact on National Institutes of Health (NIH) funding for biomedical research. After years of double-digit increases in the pool of research support, the NIH budget will now contract in real terms over the next several years. Fewer federal grants—the lifeblood of research institutions—will be awarded. At the same time, the cost of doing good science is increasing dramatically. This set of circumstances will require us to do a number of things extremely well. We must continue to attract the best scientists to Cold Spring Harbor Laboratory and maintain an environment where they can do exceptional work. At the same time, we must aggressively pursue private sources of funding for research in addition to attracting significant federal support.

Success in these areas is essential not only to the realization of the Laboratory's scientific mission, but also to the well being of our dedicated staff. Our administrative staff is lean and faces increasing demands and pressures as the scope of activity expands. The institution cannot function without this talented team of people and we must reward them appropriately. This will be accomplished only if we are successful in attracting new sources of funding. We are confident it can be done but know that it will be hard work.

Dill Ayres
Chief Operating Officer

RESEARCH

See previous page for photos of the following scientific staff.

Row 1: Izolda Mileva; Galen Collins; Ileng Ramachandran, Prakash Kumar; Maria Simone Temporal; Sabrina Boettcher, Michael Golding

Row 2: Albert Mellick; Rotem Karni; Shih-Chieh (Jack) Lin; Nishi Sinha; Hans Guido Wendel; Rebecca Ewald, Jennifer Bestman

Row 3: Chih-Chi Yuan; Xiaoyun Wu; Shweta Tyagi; ; Catherine Cormier Miguel Zaratiegui, Derek Goto; Keisha John; ; Supriya Gangadharan; Hao-Chi Hsu

Row 4: Marissa Nolan; Farida Emran; Fuqiang Geng; Fabio Nogueira; Juan Encinas; Stephen Schuck

Row 5: Elvin Garcia; Christopher Johns; Theresa Zutavern; Vincenzo De Paola

Row 6: Allen Ludwig; David Mu; Alexandra Lucs; Peter O'Brien; Daniel Nolan; Jennifer Meth

Row 7: Amitabh Mohanty; Evan Santo; Jason Williams; Maria Yuneva; Vanisree Velamoor; Pranav Sharma

CANCER: GENE EXPRESSION

Discoveries by Leemor Joshua-Tor and Greg Hannon made a significant advancement toward a comprehensive understanding of one of the most intriguing biological phenomena to be uncovered in recent years: RNA interference (RNAi). Through X-ray crystallography, Joshua-Tor and her colleagues have determined the atomic, three-dimensional structure of an RNAi component called Argonaute. When the 3-D structure of Argonaute emerged from their data and was compared to other proteins with known structures and functions, Joshua-Tor's group found that part of Argonaute was the spitting image of the "RNAse H" family of proteins, whose members were known to cut RNA. In collaboration with Greg Hannon's group, a final experiment based on the Argonaute structure clinched the case for Argonaute2 as being *the* protein that provides the Slicer activity of RNAi in mammals. Other information led Joshua-Tor's group to propose a model that explains precisely how Argonaute binds to and cuts messenger RNA during RNAi.

Winship Herr uses the herpes simplex virus to study a human protein called host-cell factor 1 (HCF-1). HCF-1 was first identified as a key factor in herpesvirus replication. However, Herr recently showed that HCF-1 is involved in other steps of the cell division process: Without HCF-1, human cells often do not complete the division process, and even if they do, the daughter cells frequently receive unequal numbers of each chromosome. This and other findings indicate that the HCF-1 protein is required for the efficient alignment and sorting of chromosomes into daughter cells during cell division.

Jacek Skowronski studies how an AIDS virus protein called Nef commandeers the protein sorting and signaling machineries of human T cells. He has recently identified several T-cell proteins that associate with Nef and is exploring how these protein–protein interactions contribute to viral pathology. Skowronski's work has revealed a potentially effective strategy for developing improved anti-HIV therapies.

Even the relatively well-understood pathways of messenger RNA biosynthesis continue to surprise scientists with new twists. Nouria Hernandez has discovered two new components of the "RNA polymerase III" transcription machinery by biochemically reconstituting the process with purified proteins. Her group's work led to the definition of a set of minimum factors required for transcription and revealed a surprising role for a protein called actin in the process. The role of actin as a component of the cell's structural scaffolding (the cytoskeleton) has long been known, making its emergence as a factor required for transcription by RNA polymerase III an intriguing discovery. Hernandez's group also discovered an unexpected role for an enzyme called casein kinase II in RNA polymerase-III-mediated transcription.

In addition to his studies of proteins involved in messenger RNA processing, Rui-Ming Xu investigates the shapes and functions of proteins that are important for the assembly and remodeling of "higher-order chromatin structure" or structures that result from the wrapping of DNA into specifically coiled architectures. Xu's group has recently used X-ray crystallography to determine the three-dimensional shapes of two proteins (Orc1-Sir1 and Sir3) that help determine chromatin structure and thereby have significant impacts on gene expression and other DNA-related processes.

Adrian Krainer's group explores how identical pre-messenger RNA molecules can be alternatively spliced to ultimately produce different proteins. Part of Krainer's work involves the study of a family of pre-mRNA splicing factors called SR proteins, which bind to specific sites on pre-mRNA and recruit the splicing machinery to those sites. Krainer and his colleagues recently discovered a role for a particular SR protein in mRNA "surveillance." They found that high levels of the SR protein promote the destruction of defective messenger RNAs, thus preventing the production of defective proteins. In other work, they characterized a novel kind of splicing mutation in the *LKB1* tumor suppressor gene that causes Peutz-Jeghers syndrome and susceptibility to cancer.

William Tansey studies a potent growth control protein called Myc. When hyperactive and/or present in excess of its normal levels, Myc contributes to cancer. Tansey and his colleagues have recently focused on a little-studied subdomain of Myc called Myc box III (MbIII). They found that MbIII is important for inducing tumor formation in a mouse model of B-cell lymphoma. Moreover, they discovered that through MbIII,

the Myc protein can impede a natural anticancer function of cells called programmed cell death. These findings establish that the oncogenic capacity of Myc is directly linked to its ability to block programmed cell death.

Michael Myers is taking the accumulated understanding of genes and proteins to the next level by identifying how proteins interact with one another to form the complex, dynamic networks that ultimately determine cell biology. His group is determining the global architecture of protein interaction networks and revealing why these networks are robust (error tolerant) and adaptable. Myers is also investigating how protein interaction networks are altered in cancer and what key molecular "nodes" of the networks might be the best targets for cancer therapy.

Kenneth Chang, research investigator, Greg Hannon's lab.

MECHANISMS OF TRANSCRIPTION

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

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 recognizes three main classes of promoter structures. Classes I and II are present in the 5S RNA and tRNA genes, respectively, and are gene-internal. Class III is present in the U6 snRNA gene and other genes and is gene-external. The class III core promoters consist of two essential elements, a proximal sequence element (PSE) and a TATA box. Until recently, the factors required for pol III transcription from these various types of promoters were poorly defined in higher eukaryotic cells. However, in the previous year, we developed a well-defined in vitro transcription system for transcription of the type-3 human U6 promoter. The system consists of recombinant snRNA-activating protein complex ($SNAP_c$), a PSE-binding factor composed of five types of subunits, which we express in insect cells with the baculovirus system; recombinant Brf2-TFIIIB, a TATA-box-binding activity composed of the TATA-box-binding protein TBP, the TFIIB-related factor Brf2, and the SANT domain protein Bdp1, all expressed in *Escherichia coli;* and a highly purified pol III complex, which we 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. The only nonrecombinant factor in the system is the pol III complex, and so we have focused on the characterization of the factors present in this complex besides pol III subunits that might contribute to transcription activity.

Besides pol III subunits, the pol III complex we purified contained spectrin, α-myosin, clathrin, α-actinin 4, HSC70, β-tubulin, β-actin, and calmodulin, as detected by mass spectrometry analysis. On the other hand, a number of other proteins such as topoisomerase 1, PC4, NF-1, the La polypeptide, and CK2 have been implicated in pol III transcription by other investigators. To determine the role, if any, of such proteins in pol III transcription, we subjected the pol III complex to one additional purification step over a "mini Q" column. We found that β-actin, β-tubulin, and calmodulin continued to copurify with pol III. Of the factors implicated in pol III transcription by others, we could detect CK2 in the pol III complex. We then set out to determine whether these two factors might have a role in pol III transcription.

CK2 is a nuclear and cytoplasmic protein kinase present in all cell types, composed of two regulatory (β) and two catalytic subunits, of which two isoforms that are 85% identical exist in human cells (α and α′). CK2 has been implicated in pol III transcription in both yeast and human cells, where it has been reported to have a positive effect on transcription. We found that CK2 is associated not only with pol III, but also with the U6 promoter in vivo as shown by chromatin immunoprecipitations. We observed that CK2 activity was required for in vitro transcription and that it was required prior to transcription initiation. This last observation, coupled with our ability to reconstitute transcription from well-defined, separate components, allowed us to ask which factor(s) needed to be phosphorylated by CK2 for active transcription. Our results revealed that it was the pol III complex itself that needed to be phosphorylated to be active. Since, in our experiment, CK2 could be active only before the start of transcription, and since once phosphorylated, pol III could direct several rounds of transcription, the phosphorylation event appeared to be a switch that somehow converted pol III from an inactive to an active state.

In its function of pol III phosphorylation, CK2 has a positive effect on transcription. In addition, however, we also found that CK2 phosphorylation of the Brf2-TFIIIB components inhibited pol III transcription, even if pol III itself was phosphorylated. We then set up to identify which of the three Brf2-TFIIIB components—TBP, Brf2, or Bdp1—was the target of this negative regulation. For this purpose, we simply treated each component separately either with CK2 followed by inhibitor, which allowed phosphorylation, or with an inhibitor followed by CK, which prevented phosphorylation, thus keeping all of the reagents in the reactions constant. In addition, we treated pol III with CK2 followed by inhibitor, since we knew CK2 phosphorylation of pol III is required for transcription, and $SNAP_c$ with inhibitor followed by CK2, since we knew that $SNAP_c$ did not need to be phosphorylated by CK2 in vitro for activity. The factors were then used for reconstitution of U6 transcription in vitro. The results were clear-cut and showed that CK2 treatment of Bdp1, but not Brf2 or TBP, inhibited U6 transcription. We then showed that Bdp1 is indeed phosphorylated by CK2, and we mapped the phosphorylation sites to one or several of five serines and threonines.

We then asked what the in vivo relevance of this negative regulation might be. We found that Bdp1 becomes highly phosphorylated during mitosis. Extracts from mitotic cells were transcriptionally inactive and could be reactivated by addition of a CK2 inhibitor and recombinant, unphosphorylated Bdp1. By fluorescent microscopy as well as through biochemical fractionation, we determined that during mitosis, Bdp1 dissociates from the chromatin, and this phenomenon was inhibited by treatment of the cells with the CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB). One model that incorporates these observations, shown in Figure 1, proposes that pol III repression during mitosis occurs in part through CK2 phosphorylation of Bdp1, which perhaps results in Bdp1 dissociation from the chromatin.

The observation that inhibition of CK2 in mitotic extracts together with addition of Bdp1 restored transcription was striking, because we had observed previously that in extracts from actively growing cells, addition of a CK2 inhibitor repressed transcription. Indeed, we then showed that in extracts from S-phase cells, which are very active for pol III transcription, addition of a CK2 inhibitor also repressed transcription. This suggests a model in which CK2 has different roles at different stages of the cell cycle, as illustrated in the figure. In M phase, it represses transcription by phosphorylating Bdp1, whereas in phases of the cell cycle when pol III transcription is active, for example, S phase, it activates transcription by phosphorylating an unknown target. Since CK2 phosphorylation of the pol III complex is required for active transcription, this unknown target may well be the pol III complex itself.

As mentioned above, another protein that we found to be associated with pol III was β-actin. β-actin has been extensively studied as a cytoplasmic cytoskeletal protein that has roles in intracellular trafficking as well as in driving changes in cellular shape associated with cell motility, cytokinesis, endocytosis, and cell adhesion. β-actin is, however, also a nuclear protein, and in this cellular compartment, it is thought to have roles in the constitution of the nucleoskeleton, in RNA processing and export, and in transcription. So, when we found β-actin tightly associated with pol III, we asked whether it might also be present on the U6 promoter in vivo. Indeed, we could localize β-actin on this promoter by chromatin immunoprecipitations. To determine the role of β-actin in pol III transcription, we then set out to identify conditions under which β-actin might dissociate from the rest of the enzyme. One of the conditions we tested was treatment of cells with the genotoxic agent methane methylsulfonate (MMS), a treatment known to inhibit pol III transcription. We found that in IMR90 cells (but not in HeLa cells), such a treatment resulted in dissociation of β-actin from the enzyme, and this enzyme missing β-actin was inactive in the reconstituted U6 transcription assay. To regain activity, we

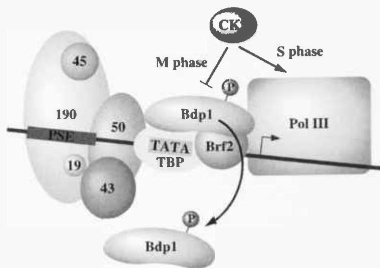

FIGURE 1 CK2: A molecular switch for the regulation of U6 pol III transcription.

then needed not only to treat pol III with CK2, but also to add recombinant β-actin. These results established a direct role for β-actin in pol III transcription. Remarkably, concomitant with our results, two reports appeared showing that β-actin is required for pol I and pol II transcriptions, and is part of the pol II initiation complex. Thus, β-actin, a protein extensively studied as a cytoplasmic cytoskeletal protein having roles in intracellular trafficking and cell shape changes, appears to correspond also to a transcription factor used by all three RNA polymerases.

PUBLICATIONS

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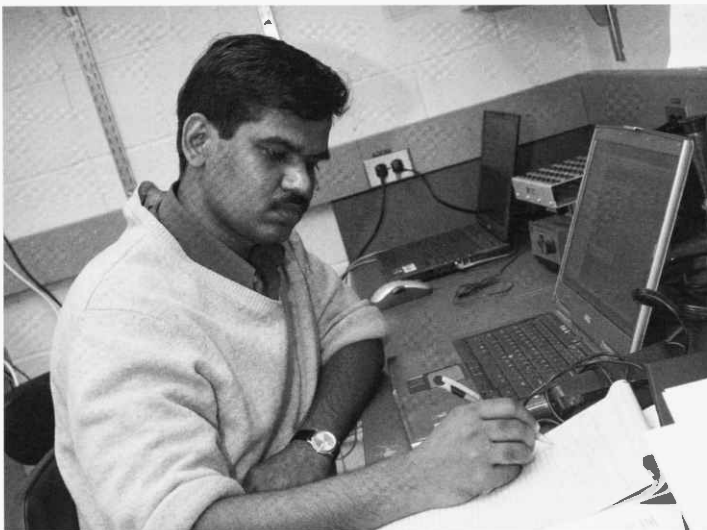

Mayilvahan Shanmugam, postdoc, Nouria Hernandez's lab.

TRANSCRIPTIONAL REGULATION

W. Herr	D. Aufiero	H. Ding	C. Papazoglu
	A. Bubulya	Y. Liu	S. Tyagi
	A.L. Chabes	M. Mangone	X. Zhao

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

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

A NONCONSERVED SURFACE OF THE TFIIB ZINC-RIBBON DOMAIN HAS A DIRECT ROLE IN RNA POLYMERASE II RECRUITMENT

This project continues to make contributions to our understanding of the mechanisms by which the basal transcriptional machinery responds to sequence-specific activators. Last year, we described how the TATA-binding protein TBP interacts with the TFIIB family of basal RNA polymerase II (pol II) and RNA polymerase III (pol III) transcription factors, of which TFIIB itself is the sole member involved in pol II transcription. TFIIB is highly conserved and is essential for accurate pol II transcription. It consists of a single polypeptide with two conserved structural domains, an amino-terminal zinc-ribbon structure ($TFIIB_{ZR}$) and a carboxy-terminal core. Thomas Tubon, in collaboration with William Tansey (CSHL), analyzed the role of the amino-terminal region of human TFIIB in transcription in vivo and in vitro. He identified a small nonconserved surface of the $TFIIB_{ZR}$ that is required for pol II transcription in vivo and different types of basal pol II transcription in vitro. Consistent with a general role in transcription, this $TFIIB_{ZR}$ surface is directly involved in recruitment of pol II to a TATA-box-containing promoter. Curiously, although the amino-terminal human $TFIIB_{ZR}$ domain can recruit both the human and yeast *Saccharomyces cerevisiae* pol II, the yeast TFIIB amino-terminal region recruits yeast pol II but not human pol II. Thus, a critical process in transcription from many different promoters—pol II recruitment—has changed in sequence specificity during eukaryotic evolution. These experiments were particularly satisfying because after studying the regulation of transcription by pol II for more than 20 years, it was the first time that our laboratory has directly addressed the role of regulatory interactions with pol II, the enzyme actually responsible for the process whose regulation we have been studying.

A SWITCH IN MITOTIC HISTONE H4 LYSINE 20 METHYLATION STATUS IS LINKED TO M-PHASE DEFECTS UPON LOSS OF HCF-1

With each year, our studies of HCF-1 grow in exciting new directions, leading to the result that, as this

year closes, our research is entirely focused on understanding the structure and function of this key regulator of the human cell cycle. HCF-1 is a highly conserved and abundant chromatin-associated protein. It exists as a heterodimeric complex of associated amino (HCF-1_N)- and carboxy (HCF-1_C)-terminal subunits that result from proteolytic processing of a large precursor protein. Studies of a mutant hamster cell line called tsBN67 have shown previously that HCF-1 has a role in both G_1-phase progression and cytokinesis. Last year, we described how the G_1-phase and cytokinesis roles of HCF-1 are performed by separate HCF-1 subunits: The HCF-1_N subunit promotes passage through the G_1 phase, whereas the HCF-1_C subunit promotes proper cytokinesis. These results suggest that HCF-1 links the regulation of exit from mitosis as defined by proper cytokinesis and the G_1 phase of cell growth, possibly by coordinating reactivation of gene expression after mitosis.

Until this year, the known role of HCF-1 in M phase, which involves both chromosome segregation in mitosis and cytoplasmic separation during cytokinesis, has been limited to the cytokinesis step. This year, in an extensive analysis of the role of the HCF-1_C subunit in M phase, Eric Julien showed that this subunit is required for multiple steps in mitosis as well. Loss of the HCF-1_C subunit leads to a switch from monomethyl to dimethyl lysine 20 of histone H4 (H4-K20) and defective chromosome alignment and segregation. Consistent with these activities, the HCF-1_C subunit can associate with chromatin independently of the HCF-1_N subunit and regulates, either directly or indirectly, the expression of the H4-K20 methyltransferase PR-Set7. Indeed, up regulation of PR-Set7 expression upon loss of HCF-1 leads to improper mitotic H4-K20 methylation and cytokinesis defects. These results establish the HCF-1_C subunit as an important M-phase regulator and suggest that H4-K20 methylation status contributes to chromosome behavior during mitosis and proper cytokinesis.

As an example of the M-phase defects that can occur in the absence of the HCF-1_C subunit, Figure 1 shows a cell that is being induced to proliferate by the HCF-1_N subunit in the absence of the HCF-1_C subunit. In this case, a cell is attempting to divide into three—rather than two!—daughter cells. We do not know whether it succeeded to divide. We look forward to performing live-cell microscopy to determine the fate of such three-way divisions of cells.

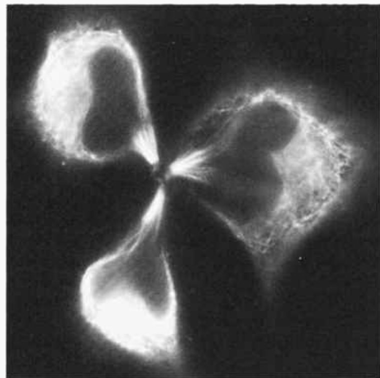

FIGURE 1 A mitotic human cell attempting to divide into three aneuploid daughters as a result of a defective *HCF-1* gene. The cell was stained with anti-tubulin antibody. The darker regions in the center of each daughter represent the nuclei. The different sizes of the nuclei are indicative of aneuploidy.

HCF-1 ASSOCIATES WITH THE MIXED-LINEAGE LEUKEMIA (MLL) PROTO-ONCOPROTEIN, A HUMAN TRITHORAX GROUP HISTONE METHYLTRANSFERASE

Last year, we reported the association of HCF-1 with a histone methyltransferase (HMT) complex related to the yeast Set1 HMT. This year, along with Akihiko Yokoyama, Michael L. Cleary, and collaborators (Stanford University), we extended the characterization of HCF-1 interaction with HMTs by showing that HCF-1 interacts with the product of the mixed-lineage leukemia (MLL) proto-oncogene. The gene that encodes MLL is mutated by chromosomal translocation in a variety of human leukemias. The MLL protein is a homolog of the *Drosophila melanogaster* trithorax protein that displays intrinsic histone methyltransferase activity and functions genetically to maintain embryonic homeobox (Hox) gene expression. Biochemical purification of MLL by A. Yokoyama and M. Cleary demonstrated that it associates with HCF-1 and the related protein HCF-2. Joanna Wysocka and Deborah Aufiero confirmed the association with HCF-1 and further showed that MLL interacts with the HCF-1 Kelch domain, the same domain responsible

for HCF-1 association with Set1. These results link HCF-1 to the maintenance of stable patterns of gene expression as well as to oncogenesis.

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Shweta Tyagi, postdoc, Winship Herr's lab.

STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

L. Joshua-Tor C. Cho T. Hu S. Smith
E. Enemark P.R. Kumar J.J. Song
H. He P. O'Farrell N. Tolia

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 to protein structure and function. Our efforts largely center on protein complexes involved in nucleic acid regulatory processes.

Mechanisms of RNAi

J.J. Song, N. Tolia, 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 approximately 20 base duplexes, with 2-nucleotide 3′ overhangs called siRNAs. The RNAi pathway also mediates the function of endogenous, noncoding regulatory RNAs called microRNAs (miRNAs). Both miRNAs and short interfering RNAs (siRNAs) guide substrate selection by similar effector complexes called the RNA-induced silencing complex (RISC). These contain single-strand 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. Argonaute proteins are defined by the presence of PAZ and PIWI domains. In a central RNAi pathway, guided by the siRNA, RISC directs the cleavage of mRNA substrates. However, the nuclease responsible for cleavage, dubbed "Slicer," has so far escaped identification.

There has been remarkable progress in unraveling the components of the RNAi machinery, but to get a true mechanistic understanding of this process, how these components fit together, and how they function, we must understand how they work at a molecular level. Therefore, we embarked on structural and biochemical studies of key proteins in the RNAi pathway, the protein-RNA and protein-protein interactions involved.

In an effort to further our understanding of the role of Argonaute in RNAi, this past year, we determined the crystal structure of the full-length Argonaute protein from *Pyrococcus furiosus* at 2.25 Å resolution (Fig. 1). The structure reveals a crescent-shaped base made up of the amino-terminal, middle, and PIWI domains. The PAZ domain is held above the base by a "stalk"-like region. The PIWI domain is similar to RNase H, with conserved active site aspartate residues, strongly implicating Argonaute as "Slicer." Several known characteristics of mRNA cleavage by RISC are consistent with an RNase-H-like enzyme. The architecture of the molecule and placement of the PAZ and PIWI domains define a groove for substrate

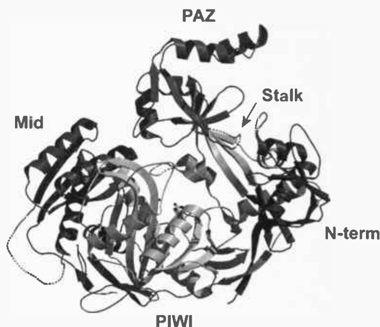

FIGURE 1 The crystal structure of Argonaute from *P. furiosus*. Ribbon representation of Argonaute showing the amino-terminal domain, the "stalk, the PAZ domain, the middle domain, and the PIWI domain.

binding. Based on the structure of PAZ with a "mini-siRNA," we could model the siRNA binding with its 3′ end in the PAZ cleft and the siRNA-mRNA double helix extending into the groove, and suggest a mechanism for siRNA guided mRNA cleavage. The notion that RISC "slicer" activity resides in Argonaute itself was tested in a mammalian system by mutational analysis of hAgo2 in collaboration with Greg Hannon's laboratory. Conserved active site aspartates in hAgo2 were altered and the mutants lost nuclease activity while retaining siRNA binding. This supports the model in which Argonaute itself functions as the Slicer enzyme in the RNAi pathway.

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

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

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

In collaboration with Arne Stenlund's group here at CSHL, we embarked on structural studies to provide high-resolution structural information about E1, its DNA-binding activity, and its assembly on DNA, which 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.

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

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RNA SPLICING

A.R. Krainer E. Allemand R. Karni F. Roca P. Smith J. Calarco L. Manche S. Shaw Q.-S. Zhang M. Hastings H. Okunola R. Sinha Z. Zhang Y. Hua

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. A brief summary of three of our recently published studies is given below.

INVOLVEMENT OF SR PROTEINS IN mRNA SURVEILLANCE

In the course of experiments aimed at addressing the relationship between premature termination codons (PTCs) and splice site selection, we uncovered a role for SR proteins in nonsense-mediated mRNA decay (NMD). SR proteins are factors required for splicing, and they also regulate alternative splicing in a concentration-dependent manner. NMD is a quality-control pathway that down-regulates mRNAs with PTCs. Model minigenes were constructed to test the effect of PTCs placed between two alternative 5′ splice sites on 5′ splice site selection (Fig. 1). In transiently transfected cells, the distal 5′ splice site was selected by default, in both the presence and absence of the PTCs (not shown). We overexpressed various SR proteins (e.g., SF2/ASF and SC35) to promote use of the proximal 5′ splice site and observed dramatically different results in the presence of PTCs, depending on which SR protein was overexpressed. As expected, both SF2/ASF and SC35 promoted use of the proximal site with the wild-type minigene to equivalent extents (lanes 3 and 5); however, when a PTC was present, no proximal splicing was detected upon SF2/ASF overexpression (lane 4), whereas SC35 overexpression resulted in proximal splicing at the same level as with the wild-type construct (lane 6). Abrogating NMD in various ways, including cycloheximide treatment, down-regulation of hUPF1 or hUPF2 by RNA interference, or expression of dominant-negative hUPF1, rescued expression of the proximal spliced mRNA with a PTC in the presence of SF2/ASF (not shown). Many natural alternatively spliced mRNA isoforms possess PTCs, and our studies suggest that the extent of their expression depends on the coexpression of the particular SR proteins that promote the appropriate alternative splicing events, as well those that do or do not trigger their degradation by NMD. PTCs also arise from nonsense and frameshift mutations, as well as from splicing mutations that change the reading frame. We found that transient overexpression of some, but not all, SR proteins strongly enhances the degradation of standard NMD substrates, i.e., constitutively spliced transcripts with a PTC. Although this effect on the NMD pathway was elicited most strongly by SF2/ASF, which is a shuttling SR protein, we also observed it with a mutant version of the protein that is retained in the nucleus.

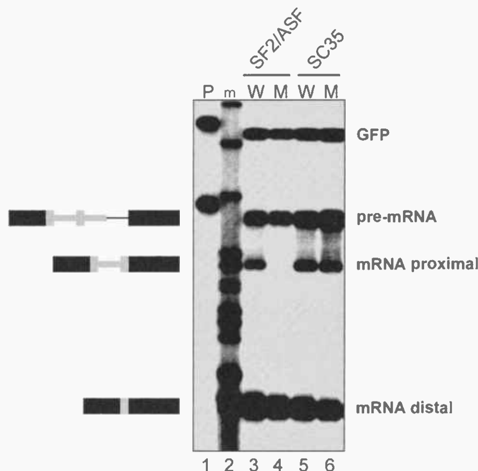

FIGURE 1 Effect of different SR proteins on sensitivity to a PTC in the context of alternative splicing. Model human β-globin genes were constructed with an internal duplication (gray) spanning the 5′ splice site in the first intron and short flanking exonic and intronic segments. The mutant construct (M) has a stop codon after each 5′ splice site, which interrupts the reading frame when the proximal (downstream) 5′ splice site is selected. The wild-type construct (W) has no premature termination codons (PTCs). HeLa cells were transfected with W and M constructs, and cotransfected with plasmids for overexpression of SF2/ASF or SC35. A GFP plasmid was also cotransfected as an internal reference for transfection efficiency, RNA recovery, and loading. The transiently expressed globin transcripts were analyzed by RPA, with a probe spanning the first exon, the duplicated region, and partial intron-1 sequences. β-globin and GFP probes were used simultaneously; undigested probes (P) are shown in lane *1*, corresponding to <1% of the amount of probe used in each RPA reaction. The bands corresponding to β-globin pre-mRNA, proximal and distal spliced mRNAs, and GFP, are indicated. For simplicity, the diagrams show only the first two exons and first intron of β-globin. (m): DNA markers.

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 now 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.

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Rotem Karni, postdoc, Adrian Krainer's lab.

PROTEOMICS

M. Myers R. Bish L.L. Schmidt
G. Pegoraro C. van der Meijden
D. Perkowski

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

The goal of our laboratory is to understand how proteins and protein complexes regulate cellular behavior. In the environment of a cell, almost all of the proteins can be found in a highly interactive network. 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, as 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 medium 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). Initially, we incorporated octadecyl (C18) or octyl (C8) functional groups into our monoliths in order to create a reverse-phase resin, and we are currently working on producing monoliths with additional functionality, such as those that can be used for ion-exchange chromatography.

A significant loss in signal occurs due to poor ion transfer between the nanocolumns and the mass spectrometer. We have optimized a number of parameters to improve our electrospray interface. In collaboration with Patrik Kallback (GE Healthcare), we have developed a novel high-voltage interface that maximizes electrical contact with the column effluent, resulting in a more robust ionization at lower voltages. In addition, this interface is resistant to fouling. One of the drawbacks of chromatography-coupled electrospray interfaces is that the voltage must be adjusted during the analysis in order to maintain a single, stable Taylor cone. To improve Taylor cone stability, we have adapted a static nanospray chip to our system. Importantly, the nanospray chip improves the potential difference between the column effluent and the mass spectrometer, resulting in stable Taylor cone formation throughout the chromatography run.

Monitoring Posttranslational Modifications

L.L. Schmidt, D. Perkowski

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

Therefore, efficient identification of phosphorylated proteins, as well as mapping the specific 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 MS, (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 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 phosphoric acid and/or instability of the phosphopeptides in the gas phase. In fact, the suppression due to phosphorylation can be alleviated by altering the ionization conditions to improve volatility and stability during the ionization process.

"Small World" Proteomics

C. van der Meijden, G. Pegoraro [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, as essential proteins are highly enriched in the pool of highly connected proteins. Importantly, not all highly connected genes are essential, and we are focusing on trying to understand the differences between these proteins and those which are essential. The overall goal of this study is to understand how these protein networks provide adaptability to genetic alterations, such as loss or duplication of a node (gene) that occurs during tumorigenesis. Importantly, nongenotoxic stresses, such as high salt or nutrient depletion, increase the spontaneous mutation rate in yeast and in a number of other organisms. The stress-induced mutations are believed to provide a mechanism for rapid adaptation to changing environmental conditions. We are currently investigating whether the essential genes are protected from these mutations, and we have discovered that the essential and nonessential genes have different genomic distributions.

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

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CELL SIGNALING IN HIV PATHOGENESIS

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Our interest lies in understanding the molecular mechanisms underlying the pathogenesis of AIDS and, in particular, understanding the functional consequences of interactions between viral proteins and the cellular regulatory machinery. The main focus of our research is to understand the functions of Nef, a regulatory protein of human and simian immunodeficiency viruses (HIV and SIV) that is an important determinant of virulence. We directed a major effort toward the identification of mechanisms and downstream effectors that mediate the effect of Nef on protein sorting and signal transduction machineries. and our experiments have been focused in two areas. First, we 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 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. We have also begun to verify selected novel interactions and to address their relevance to previously known and possibly novel functions of Nef, as well as to AIDS pathogenesis. Second, we continued studies to address the relevance of individual Nef activities for rate of progression of lentiviral replication in vivo. To this end, we completed the studies of the impact of down-modulation of class I major histocompatibility complex (MHC) by Nef on antiviral immune response and found that this Nef function indeed limits in important ways the generation of virus-specific $CD8^+$ T cells in the infected host.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL Nef EFFECTORS

Nef is a small adaptor protein that acts through associations with large protein complexes. We recently purified to near homogeneity Nef and its associated protein complexes from T-cell and monocyte lines stably expressing Nef using an immunoaffinity purification protocol and identified their components by mass spectroscopy. This approach allowed us to detect a series of polypeptides with apparent molecular masses ranging from 20 kD to more than 250 kD that copurified with HIV-1 and SIV Nef proteins. Next, we determined their sequences by mass spectroscopic analysis, in collaboration with Michael Myers here at the CSHL Protein Chemistry Shared Resource. This led to the identification of several common polypeptides associated with both 7.AH Nef and 239.AH Nef, including subunits of the DOCK2-ELMO1-Rac2 complex and additional polypeptides involved in control of various housekeeping functions of a cell. During the last year, we have continued to study these associations. We have also characterized in detail the complex comprising Nef and isoforms of *N*-myristoyltransferase (NMT).

Nef ASSOCIATES SELECTIVELY WITH ISOFORM 1 OF NMT

All known functions of Nef require that it be myristoylated at its amino terminus. This reaction is catalyzed by NMTs, which transfer myristate from myristoyl coenzyme A (myristoyl-CoA) to the amino-terminal glycine of substrate proteins. Two NMT isoforms (NMT-1 and NMT-2) are expressed in mammalian cells. Interestingly, NMT-1 but not NMT-2 was identified as one of relatively abundant cellular proteins that associated with both HIV-1 and SIV Nef. The Nef-NMT-1 complex is most likely a transient intermediate of the myristoylation reaction of Nef because its formation requires the amino-terminal myristoylation signal in Nef and is modulated by agents that affect the size of the myristoyl-CoA pool in the cell. We also examined two other proteins that bear an amino-terminal myristoylation signal, HIV-1 Gag and Hck protein tyrosine kinase. Surprisingly, we found that Gag bound preferentially the NMT-2 isoform, whereas Hck bound mostly to NMT-1. Recognition of different NMT isoforms by these viral and cellular substrate

proteins suggests nonoverlapping roles for these enzymes in vivo and reveals a potential for the development of inhibitors that target the myristoylation of specific viral substrates more selectively.

ANTIVIRAL IMMUNE RESPONSE AND SIV VIRULENCE

Presentation of antigens derived from viral proteins expressed in infected cells by class I MHC molecules to competent effector T cells is the key event required for the recognition and elimination of infected cells by the immune system of the host. We showed previously that Nef proteins down-regulate expression of class I MHC complexes by inducing their endocytosis from the cell surface. Down-regulation of class I MHC expression could be very important for the establishment of persistent SIV/HIV infection once strong antiviral responses develop in the infected host, but whether it is so has been a subject of controversy.

To address directly the role of this Nef function as well as a broader issue of the role of antiviral immune response in immunodeficiency virus infection and progression to AIDS, we studied in detail class I MHC down-regulation by Nef protein derived from a pathogenic SIVmac strain. First, we identified mutations in the carboxy-terminal domain of SIVmac 239 Nef that selectively disrupt class I MHC down-regulation, having no detectable effect on other functions of Nef such as the down-regulation of CD4 and CD3 surface expression, and the stimulation of SIV virion infectivity and induction of SIV replication from peripheral blood mononuclear cells (PBMCs) infected in the absence of stimulation. Second, we constructed several mutant Nef proteins with small deletions in the carboxy-terminal region (NefΔ239-240, Nef238stop/fs/fs). These mutations still selectively disrupted only class I MHC down-regulation, but are less likely to revert in vivo than point mutations. These mutants turned out to be powerful reagents to study the importance of class I MHC down-regulation for SIV replication in the context of a functional immune system in rhesus macaques. Then, to define the effect of these selective mutations on anti-SIV immune response we collaborated with Dr. R.C. Desrosiers (New England Primate Center, Harvard) who infected rhesus monkeys with SIVmac viruses carrying nefΔ239-240, nef238stop/fs/fs and, as controls, SIVmac viruses containing wild-type *nef*, or a *nef* mutant with a 181-nucleotide deletion that abrogates all known functional activities of Nef.

Nef LIMITS ANTIVIRAL RESPONSE BY CD8⁺ T CELLS

The ability of $CD8^+$ T lymphocytes from infected monkeys to react with antigenic peptide and an MHC I tetramer corresponding to the Mamu-A*01-restricted Gag181-189 epitope CTPYDINQM was monitored. The four monkeys infected with the SIV strains carrying the MHC-selective *nef* mutations exhibited higher levels of $CD8^+$ T-cell cellular responses than the four monkeys infected with the parental wild-type SIV239 at 4–14 weeks after infection. These differences were statistically significant. Differences became less discernible by 16–20 weeks postinfection.

$CD8^+$ T-cell responses were significantly higher in monkeys that received the MHC-selective mutant SIVs than in monkeys that received parental SIV239, from 4 to 14 weeks postinfection. Stronger $CD8^+$ antiviral responses would be expected to translate into lower viral loads, but we were unable to demonstrate any statistically significant differences in viral loads among the groups, excluding of course the SIV*Δnef* animals. It seems likely that the evolution of restored MHC down-regulating activity minimized the magnitude and duration of any effects of the mutations on viral load. The number of monkeys used was necessarily limited, and significant differences may have been observed with much larger numbers of monkeys.

A controlling immune response is likely to consist of multifactorial components; the effects of the MHC activity of Nef influenced the levels of only one of these multifactorial components: virus-specific $CD8^+$ cellular responses. SIV destruction of virus-specific $CD4^+$ helper cells may have limited the effectiveness of increased numbers of virus-specific $CD8^+$ T cells that resulted from the loss of MHC down-regulating activity. Nonetheless, although we were not able to demonstrate significant differences in viral load, the selective pressure for sequence change to restore MHC down-regulating activity clearly indicates that the absence of this activity did serve to limit the replication of the virus at least somewhat.

CLASS I MHC DOWN-REGULATION BY MUTANT Nef PROTEINS IS RESTORED THROUGH SELECTION OF SECOND-SITE COMPENSATORY MUTATIONS

To address the normalization in numbers of Gag-specific $CD8^+$ T cells at later timepoints, we next determined the evolution of sequence changes in *nef* over time. *nef* sequences present at 16 and 32 weeks postinfection were amplified by polymerase chain reaction

(PCR) of DNA from cells infected with recovered virus. Little variation was observed in the week-16 samples from the two control animals infected with the wild-type virus. In contrast, greater levels of variation were observed in the monkeys infected with the MHC-selective mutants at 16 weeks. In all clones analyzed at 16 and 32 weeks after infection in our current study, the difficult-to-revert NefΔ239-240 and Nef 238stop/fs/fs mutations were retained, forcing the virus to try to compensate by changing residues elsewhere in the Nef protein. The changes in Nef progressively increased the ability to down-regulate MHC from weeks 0 to 16 to 32 such that by week 32 the ability to down-regulate MHC class I was similar to that of the parental SIVmac 239 Nef. Based on the large number of sequence changes and the time required to restore full MHC down-regulating activity, the virus was clearly under strong selective pressure over a prolonged period to restore the activity; this provides unambiguous evidence for the contribution of MHC down-regulation to the ability of SIV to replicate in monkeys. The ability to restore the activity by sequence changes quite distant in the linear sequence is testimony to the remarkable flexibility and multiplicity built into the Nef protein structure.

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TRANSCRIPTIONAL CONTROL AND THE UBIQUITIN-PROTEASOME SYSTEM

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Correct regulation of gene expression is mediated, in part, by the action of sequence-specific transcription factors that bind to control elements within genes and stimulate the recruitment and activity of RNA polymerase II (pol II). The importance of transcription factors to the control of normal cellular processes is evidenced by the fact that many transcription factors are the products of oncogenes and tumor suppressors. Oncoprotein transcription factors such as Myc, for example, drive the expression of a battery of genes required for cell cycle progression and cell growth. Ectopic expression of Myc leads to uncontrolled proliferation and eventually tumorigenesis, and deregulated Myc levels are commonly found in a variety of human cancers. Indeed, it has been estimated that fully one third of cancer deaths in the United States can be attributed to aberrant expression or activity of the Myc protein.

Consistent with their potent growth-promoting properties, the activity of proteins such as Myc are tightly limited within the cell. Perhaps one of the most striking ways in which transcription factor function is restricted is via their rapid proteolysis. Indeed, transcription factors—most notably those involved in responding to a transient signal from outside the nucleus—are generally unstable proteins that are destroyed by the ubiquitin (Ub)-proteasome system. Our research is targeted toward understanding how transcription factors such as Myc are destroyed by the Ub-proteasome system, and the significance of this destruction to the regulation of gene expression.

CONNECTION BETWEEN TRANSCRIPTION FACTOR ACTIVITY AND DESTRUCTION

Although transcriptional activation and Ub-mediated proteolysis are two processes that have apparently very little in common, research from a number of laboratories, including our own, has revealed that these two processes come together to control gene activity. Our efforts in this area began with the observation that, within Myc, the element that signals Myc ubiquitylation (the so-called "degron") overlaps *precisely* with the domain that activates transcription (the transcriptional activation domain, or TAD). Subsequent studies have revealed that the overlap of TADs and degrons occurs in most unstable transcription factors, that the relationship between these elements is intimate, and that, in some cases, the ability of an activator to engage the Ub-proteasome system is essential for transcription activation. Together with other findings demonstrating that Ub ligases can function as transcriptional coactivators, that some general transcription factors have Ub ligase activity, and that components of the proteasome have a nonproteolytic role in transcriptional elongation and chromatin modifications, these observations suggest a deep mechanistic connection between the transcription and ubiquitin systems that we are anxious to explore.

Research in our laboratory is divided into two parallel, but complementary, strands. To understand the basic mechanisms through which Ub-dependent transactions regulate gene expression, we study model transcription factors in the yeast *Saccharomyces cerevisiae*. To probe the significance of the Ub-transcription connection to mammalian cell growth control, we ask how the basic mechanisms we learn about from our yeast studies apply to regulation and activity of the Myc oncoprotein.

TWO DISTINCT MODES OF UB-MEDIATED PROTEOLYSIS DIFFERENTIALLY REGULATE THE YEAST TRANSCRIPTION FACTOR Gal4

Our previous studies have revealed that some transcription factors must be ubiquitylated to activate transcription. We have interpreted this requirement to be a type of a "licensing" mechanism, whereby the activity of transcription factors is limited to a narrow window of time between when it is ubiquitylated and when it is ultimately destroyed by the proteasome. Interestingly, in this scenario, Ub ligases—the enzymes that mediate transcription factor ubiquitylation—serve as essential

transcriptional coactivators. The concept that a Ub ligase can stimulate the activity of a protein that it ultimately destroys is very different from traditional views, where the Ub system acts to terminate protein function when no longer required. Although the licensing model accounts for the close relationship between transcription factor activity and destruction, and for the growing number of Ub ligases that have been shown to have transcriptional coactivator function, this model cannot explain all regulation of transcription factors by the Ub system. The tumor suppressor transcription factor p53, for example, is ubiquitylated via the Mdm2 Ub ligase, and it is clear that Mdm2 *antagonizes* p53 activity. Similarly, we have shown that Myc activity is stimulated by a Ub ligase called Skp2, whereas other groups have shown that the Ub ligase Fbw7 destroys Myc and blocks its function. Clearly, therefore, the regulation of transcription factors by components of the Ub system is complex.

To resolve some of this complexity, we asked how the native yeast transcription factor Gal4—which activates genes required for galactose utilization—interacts with the Ub system under conditions in which it is inactive (growth in media containing raffinose as a carbon source) versus active (galactose carbon source). Our hypothesis was that the Ub system may regulate transcription factors differently depending on their activity. To this end, we developed a method that would allow us to hone in on transcriptionally active populations of Gal4 protein. We took advantage of observations made by the Ptashne, Hopper, and Sadowski laboratories that revealed that Gal4 is phosphorylated by pol-II-associated kinases as a *consequence* of activating transcription. Although these phosphorylation events are not themselves required for transcription, we reasoned that we could use them—and their characteristic effects on the electrophoretic mobility of Gal4—to identify pools of Gal4 that we could verify had functionally interacted with the transcription machinery. Under inactive (raffinose) conditions, Western blotting reveals two Gal4 isoforms, termed a and b (Fig. 1A). Under active (galactose) conditions, isoforms a and b persist, but a new isoform, termed c, appears. Gal4c formation is mediated by basal factor-associated kinases and (unlike Gal4a and b) is absolutely dependent on the ability of Gal4 to activate transcription. We conclude that Gal4c corresponds to the pool of Gal4 that either is activating or has activated transcription. Gal4a/b, in contrast, correspond to inactive Gal4 protein. Having distinguished these populations of Gal4 in this way, we asked how each Gal4 population interacts with the Ub system.

When yeast are grown under noninducing (raffinose) conditions, Gal4a/b are moderately ustable, with an approximate half-life of 20 minutes (Fig. 1B). The turnover of Gal4a/b is mediated by the Ub system and is critically dependent on a Ub ligase called Grr1. In the absence of Grr1, Gal4a/b are stable, and Gal4 protein accumulates. Interestingly, disruption of Grr1 stimulates Gal4 activity, resulting in ectopic *GAL* gene activation, even in raffinose media (Fig. 1C). These findings suggest that Grr1 normally acts to restrict Gal4 function and are consistent with traditional views of how the Ub system controls protein activity (e.g., the p53-Mdm2 relationship).

When yeast are grown under inducing (galactose) conditions, however, a different pattern of behavior is observed (Fig. 1D). Gal4a/b are stabilized, whereas the active Gal4c isoform is extremely unstable, turning over with a half-life of just a few minutes. Destruction of Gal4c is mediated by the Ub-proteasome system and is dependent on a Ub ligase called Dsg1. In the absence of Dsg1, Gal4c is stable, and the active isoform accumulates. Our studies have shown that Dsg1 is essential for Gal4 ubiquitylation under galactose growth conditions and that Dsg1 binds to chromatin at sites overlapping with the Gal4 protein, suggesting that Dsg1 mediates Gal4 ubiquitylation and destruction at target gene promoters. The role of Dsg1 in GAL gene activation is intriguing: In the absence of Dsg1, Gal4 can stimulate transcription of its target genes, but the resulting RNAs are not translated (Fig. 1E–F). The translational defects of these RNAs are related to defects in the postinitiation phosphorylation of RNA pol II, and the inability of pol II complexes to mature and to functionally associate with RNA processing machinery (e.g., capping enzyme). These findings are consistent with the licensing model, because Dsg1 is functioning as a Gal4 coactivator, and argue that Ub-mediated destruction of Gal4 (and possibly other components of the transcriptional apparatus) is required to drive important transitions that define different steps in the transcription process (initiation, elongation, mRNA processing, etc.).

In summary, these studies of Gal4 reveal that a single transcription factor can be dealt with by the Ub system in very different ways, depending on whether it is activating transcription. Furthermore, our results demonstrate that the Ub system regulates an unexpected step in transcription, where RNAs are signaled to become functional messenger RNAs. Finally, our findings imply that Ub-mediated proteolysis may have important roles in other transitions required for the "transcription cycle." As pol II transcribes genes, its complement of interacting proteins must change not

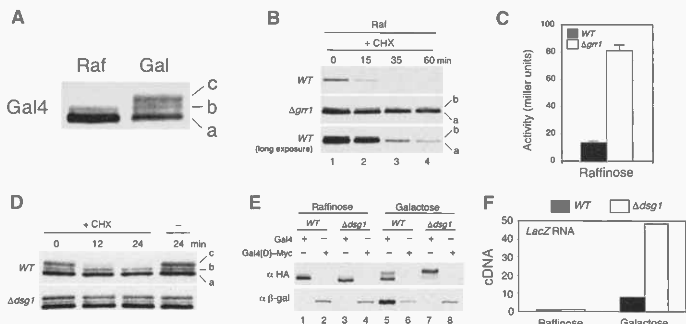

FIGURE 1 Two modes of proteolysis regulate Gal4 function. (*A*) Tracking active pools of Gal4 protein. Western blot, showing patterns of Gal4 phosphoisoforms that can be observed in media containing either raffinose (Raf; inactive) or galactose (Gal; active) as a carbon source. Formation of the Gal4c isoform is tightly connected to transcriptional activation. (*B*) The F-box protein Grr1 is required for Gal4a/b turnover in raffinose media. Wild-type or congenic grr1-null yeast were grown in raffinose media, cyclohexamide was added to inhibit protein synthesis, and protein samples were taken at the indicated time points ("cyclohexamide chase"). Gal4a/b disappear quickly in wild-type yeast, but persist in the *grr1*-null cells. (C) Deletion of *Grr1* results in ectopic activation of a *GAL1-lacZ* reporter gene. β-galactosidase activity was assayed from wild-type and Δ*grr1* yeast growing in raffinose-containing media. (*D*) The F-box protein Dsg1 is required for Gal4c turnover in galactose media. Cyclohexamide chase. In galactose media, Gal4a/b are stable (*top panel*), but Gal4c disappears quickly following inhibition of protein synthesis. Gal4c turnover depends on the presence of the *Dsg1* gene. (*E*) Deletion of *Dsg1* results in a failure to functionally activate a *GAL1-lacZ* reporter gene. Western blotting was used to detect β-galactosidase protein induced by either Gal4 (*odd-number lanes*) or a synthetic activator in which the Gal4 TAD was replaced with the Myc TAD (Gal4[D]-Myc; *even-number lanes*). In the absence of *Dsg1*, Gal4c accumulates (*top panel lane 7*; α-HA), but there is no detectable induction of β-galactosidase protein (*lower panel lane 7*; αβ-gal). The Gal4-Myc activator is unaffected by loss of *Dsg1*. (*F*) Deletion of *Dsg1* results in an accumulation of RNAs from the *GAL1-lacZ* reporter. RNA was isolated from the same cells assayed in *E*, and *lacZ* RNAs quantified by reverse transcription, real-time polymerase chain reaction (PCR). Despite the failure to detect β-galactosidase protein in Δ*dsg1* cells, *lacZ* RNAs accumulate to high levels in these yeast.

only during the elongation checkpoint, but also during events involved in termination and 3′-end processing, and when pol II encounters a site of DNA damage. The involvement of proteolysis in these transitions would not only actively drive disassembly of these complexes, but would ensure that events important for RNA processing and polymerase disengagement occur in an irreversible manner. Perhaps, therefore, as in the cell cycle, proteolysis provides an unequivocal signal of directionality to the transcription process.

REGULATION OF Myc ONCOPROTEIN FUNCTION BY THE UB SYSTEM

The intimate connection between transcription factor activity and destruction provides us with a unique opportunity to learn more about how Myc protein functions. Our thesis is that, if activity and destruction *are* linked, then we should be able to use one of these activities to learn about the other. Recently, for example, we identified a novel element in Myc that is required for its rapid proteolysis. The connection between transcription factor activity and destruction prompted us to ask whether this element is also required for Myc function. In collaboration with the Lowe laboratory here at CSHL, we showed that this element is indeed required for cellular transformation by Myc and appears to function by attenuating the natural ability of Myc to induce apoptosis. We have now identified several proteins that interact specifically with this region of the Myc molecule and are pursuing the connection of these proteins to the regulation of apoptosis. In a similar vein, again in collaboration with the Lowe laboratory, we have examined the activity of various cancer-associated Myc mutants that we showed a number of years ago block Myc destruction

at a *postubiquitylation* stage. Curiously, these mutants allow Myc to transform cells without the requirement for loss of p53, suggesting that blocking Myc destruction does not simply lead to overexpression of Myc protein, but rather results in a qualitative shift in Myc behavior. The ability of stabilized forms of Myc to circumvent the apoptotic response further emphasizes the intimate connection between Myc activity and turnover, and suggests that further study of Myc destruction will reveal fundamental aspects of how Myc promotes oncogenesis.

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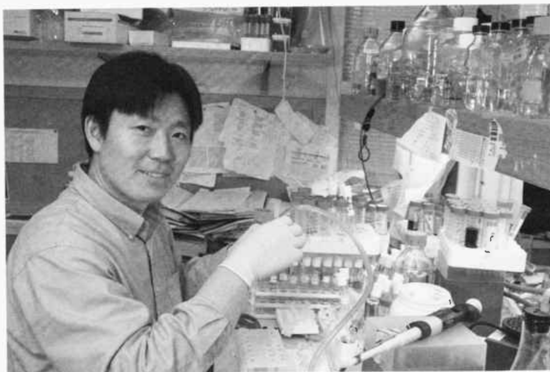

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STRUCTURAL BIOLOGY

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Our research is directed toward understanding the molecular mechanisms by which eukaryotic genes are regulated at both the transcriptional and posttranscriptional levels. We continue to focus our research on structural studies of proteins involved in mRNA processing and proteins that are important for the assembly and remodeling of high-order chromatin structures. During 2004, we determined the structures of an Orc1p-Sir1p complex and the amino-terminal domain of Sir3p, which are part of our systematic investigation of the molecular mechanism of epigenetic control of gene expression in yeast.

In the budding yeast *Saccharomyces cerevisiae*, cryptic mating-type genes, *HML* and *HMR*, are epigenetically silenced. The silent *HM* loci are flanked by specific *cis*-acting DNA sequences called the E and I silencers, which contain binding sites for DNA-binding proteins, the origin recognition complex (ORC), Rap1p and Abf1p. Silencing at the *HM* loci also requires four silent information regulator (SIR) proteins, Sir1p-4p. Orc1p, the largest subunit of ORC, interacts with Sir1p, and this interaction is important for the establishment of silencing. In collaboration with Bruce Stillman, we have previously solved the crystal structure of the Sir1p-interacting amino-terminal domain of Orc1p (Orc1N), which contains a bromo-adjacent homology (BAH) domain found in many chromatin-associated proteins.

We have determined a 2.5 Å cocrystal structure of the amino-terminal domain of Orc1p in complex with the Orc1p-interacting domain (OID) of Sir1p. The structure reveals that Sir1p OID has a bilobal structure—an α/β amino-terminal lobe and a carboxy-terminal lobe resemble the fold of the Tudor domain "royal family" members, which have been implicated in binding histone tails. The amino-terminal lobe of Sir1p binds in a shallow groove between a helical subdomain and the BAH domain of Orc1p (Fig. 1). The structure is useful for understanding the specificity of Orc1p-Sir1p interaction and the mechanism for the establishment of silencing.

We have also determined the structure of the amino-terminal domain of Sir3p (Sir3N), which shares significant sequence homology with Orc1N. The two homologous domains have similar but distinct functions in transcriptional silencing, and the mechanism by which Sir3N functions in silencing is not understood. The 1.9 Å resolution structure reveals that Sir3N shares a very similar overall structure with that of Orc1N; nevertheless, the amino-terminal tails of Sir3N and Orc1N have significantly different conformations, despite 100% sequence identity of the first eight residues between the two proteins. In the Orc1N structure, the amino-terminal tail is involved in interacting with neighboring molecules. This region of Sir3N is important for silencing and the same region is also involved in protein-protein interactions between the two molecules within the same asymmetric unit. It is possible that homodimeric interaction between the two Sir3 amino-terminal domains is important for silencing.

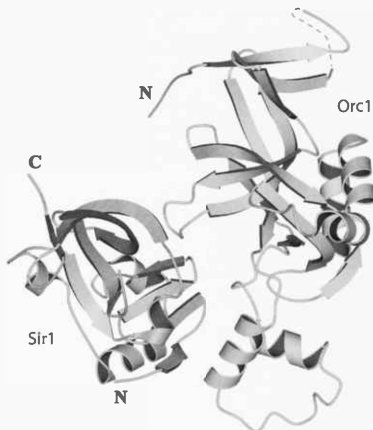

FIGURE 1 Crystal structure of the Orc1N-Sir1p OID complex. The structures are shown in a ribbon representation.

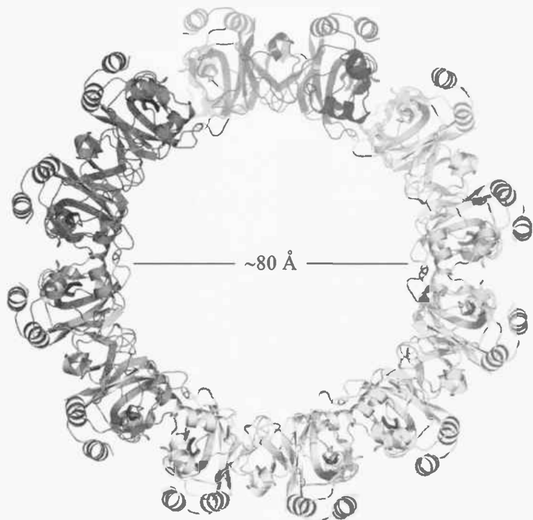

FIGURE 2 Sir3N packs into a super-helical polymer in the crystal—a view along the super helical axis.

In the crystal lattice, the Sir3N dimer in the asymmetric unit packs with symmetry-related molecules to form a contiguous left-handed super helix. Figure 2 shows a view of the crystal packing along the super-helical axis. Three "strands" of parallel protein dimers wrap around each other to form a tubular architecture. The inner diameter of the super helix is approximately 80Å. The pitch of the super helix is approximately 197 Å. Although the organization of the super helix in the crystal lattice is under a rather artificial condition of crystal packing, nevertheless, packing interactions can sometimes stabilize weak and transient interactions that might be difficult to detect in solution. It is interesting to note that the minimal diameter of a nucleosome core particle is about 85–90 Å. Considering the physiological function of Sir3p in spreading along the chromatin, it is possible that Sir3p coats the nucleosomes within the silent chromatin domain in a fashion similar to the one observed in the crystal packing.

In addition to the structural characterization of yeast silencing protein complexes, we are continuing our analyses of RNA-splicing factors and various multiprotein complexes of the spliceosome. Our overall goal is to determine the structures of a number of spliceosomal components that will enable us to assemble the structures together to gain insights into structure and function of the large and complex splicing "machine."

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CANCER: GENETICS

Despite tight control of DNA replication, errors inevitably creep in. Most are corrected, but some go unrepaired and lead to mutations or changes in the cell's genetic code. Eli Hatchwell is using genomic screening methods to compare large chunks of human DNA to detect the rare mutations that occur during the cell divisions that produce eggs and sperm. These mutations lead to nonheritable or "sporadic" genetic disease in offspring. Traditionally, these mutations have been difficult to detect, but Hatchell's method has been successful in uncovering these rare events. Understanding such sporadic genetic diseases helps researchers understand the origin of more common disorders.

The gain ("amplification") or loss ("deletion") of DNA segments has emerged as a major driving force of cancer. Michael Wigler and Rob Lucito have developed a powerful method for measuring such differences in copy number between normal and cancer cells. By using their method, called representational oligonucleotide microarray analysis (ROMA), Wigler and Lucito have identified a number of genes that have previously undiscovered roles in the biology of cancer. For example, to gain a better understanding of how primary tumors spread or metastasize to other regions of the body (a frequently lethal process), Lucito is using ROMA to compare the DNA of premetastatic and postmetastatic ovarian tumors. In addition to their findings from studying breast, prostate, colon, pancreatic, and other cancers, Wigler, Lucito, and their colleagues have identified a region of DNA that is specifically amplified in half of the postmetastatic ovarian cancers they analyzed. This observation identifies a prime molecular target for therapies to halt the spread of not only primary ovarian tumors, but also of other cancers.

By using a technology on which ROMA is based, Scott Powers and his colleagues previously identified a gene—called *PPM1D*—that was amplified and upregulated in 15% of the breast cancer specimens they analyzed. Whether *PPM1D* had any role in a larger patient population was unknown. Recently, however, Powers has shown that Ras- and ErbB2-induced breast tumor formation is significantly impaired in *PPM1D*-deficient mice. This finding suggests that if particular challenges can be overcome, small molecule or other inhibitors of *PPM1D* could form a new class of broad-spectrum cancer therapies.

The *p63* gene is related to *p53*—a tumor suppressor gene implicated in more than half of all human cancers. In an effort to investigate the role of *p63* both in normal development and tumor formation, Alea Mills has developed a series of mouse models that provide systems for investigating *p63*'s role in vivo. The models her group has generated include animals with the complete absence of *p63*, those in which the timing and tissue specificity of *p63* expression can be precisely controlled, and others with specific *p63* amino acid mutations that correlate with "hot-spot" mutations in *p53* in human cancers. These animal models are a powerful tool for exploring the role of *p63* in development, stem cell maintenance, and cancer. In other work that also uses animal models for studying cancer biology, Mills is deleting specific regions of the mouse genome that correspond to a region of the human genome that is frequently deleted in a variety of cancers.

RNA interference (RNAi) has emerged as a widespread biological regulatory mechanism, as a powerful tool for both basic and applied research, and as a therapeutic strategy of enormous potential. In organisms from fungi and flies to plants and humans, RNAi has an essential, multifaceted role in controlling gene expression. Greg Hannon, a pioneer of RNAi research, continues to identify new components of the RNAi pathway and to determine how they function. Recently, he and his colleagues have begun to unravel how "microRNAs"—small, noncoding RNAs that regulate gene expression through RNAi—are matured. This involves the action of the "Microprocessor complex" and a newly discovered component of that complex, called Pasha. In other work, a collaboration between Hannon and Leemor Joshua-Tor (see below) led to the identification of Argonaute2 as the key catalytic component of RNAi. Finally, Hannon has collaborated with Dick McCombie here at CSHL, Steve Elledge of Harvard, and colleagues at Merck and OSI Pharmaceuticals to develop large-scale RNAi libraries that will eventually enable scientists to shut off virtually every gene in the human and mouse genomes for a wide variety of cancer research and other biomedical applications.

In part of his recent work, Scott Lowe used RNAi to study the *p53* tumor suppressor pathway. His group showed that suppressing the production of "PUMA" (a target of *p53* action) by RNAi dramatically accelerated the development of lymphoma in mice. These findings indicate that despite the large number of genes controlled by *p53*, disruption of a single *p53* effector can largely disable *p53* tumor suppressor functions in some contexts. In other research, Lowe's group showed that a particular two-drug combination therapy leads to the complete remission of a mouse model of B-cell lymphoma in all of the treated animals. Because the therapy targets pathways that are involved in the majority of human tumor types, it establishes a new paradigm for treating many forms of human cancer and has entered clinical trials.

In addition to its use as a research tool, RNAi is showing promise as a potential therapy. Vivek Mittal studies "angiogenesis" or the recruitment of a new blood vessel supply to the sites of tumors. Mittal's group has demonstrated that RNAi directed at a key factor involved in blood vessel formation can delay tumor growth. They are examining the molecular pathways that are involved in the mobilization of cells that form blood vessels and are hopeful that their approach will aid the development of anti-angiogenesis therapies for cancer and other diseases.

Programmed cell death or "apoptosis" is carried out by specialized molecular machinery that rids precancerous cells from the body. One hallmark of cancer is that the apoptosis machinery is inactivated by mutation in a tumor cell. Selectively restoring the ability of cancer cells to kill themselves via apoptosis is therefore an attractive therapeutic strategy. Yuri Lazebnik is exploring the possibility that cancer cells might in fact carry the seeds of their own destruction on the basis of his paradoxical discovery that under certain conditions, some oncogenes can trigger programmed cell death. His group is continuing to investigate therapies for cancer based on selectively restoring apoptosis in cancer cells.

Masaaki Hamaguchi is investigating the function of a gene called *DBC2* (for *d*eleted in *b*reast *c*ancer) that he previously discovered was missing from or inactive in a large proportion of breast and lung cancers. By both decreasing or increasing production of the Dbc2 protein in human cell cultures, Hamaguchi and his colleagues found that two cellular networks react significantly to changes in Dbc2 protein levels. One of the networks regulates growth through cell cycle control and programmed cell death. The other network is related to cytoskeleton and membrane trafficking. These findings reveal new targets for treating cancers that lack Dbc2 protein expression.

ANALYSIS OF A TUMOR SUPPRESSOR GENE

M. Hamaguchi S. Goldman J.L. Meth V. Siripurapu
E. Greenberg C.B. Meyer C.A. Sorensen
N. Kobayashi R. Shudo

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) that is likely to be a tumor suppressor gene for sporadic breast cancer. Activation of *DBC2* in a breast cancer cell line resulted in growth arrest of the cells, whereas naturally occurring *DBC2* mutants do not suppress tumor growth. The ultimate goal of our research is elucidation of the tumor suppression mechanisms of *DBC2* and development of new cancer therapy based on the findings.

DBC2 and Gene Expression

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

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

One of our approaches is a microarray analysis that allows us to monitor a large number of genes at the same time. DNA microarrays contain thousands of DNA probes in a small area, e.g., 1 inch x 2 inch, on a glass slide. When a sample DNA is incubated with a microarray chip, DNA fragments in the sample hybridize with corresponding probes. As a result, the signal intensity of a probe represents the amount of the matching sequences in the sample. When cDNA from a specimen is used as a sample, gene expression levels can be profiled. This is particularly useful when we analyze global changes in gene expression.

Using methods described above, *DBC2* expression was manipulated and gene expression profiles were examined. Approximately 700 genes were found to be affected 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 have successfully discovered four cellular pathways that are significantly disturbed by *DBC2* expression (Table 1). They are related to apoptosis, cell cycle control, membrane trafficking, and cytoskeleton (Siripurapu et al.). 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–Protein Interaction

M. Hamaguchi, N. Kobayashi, R. Shudo,
S. Goldman [in collaboration with N. Sato,
Tokyo Metropolitan Institute of Medical Science]

In addition to the global approach described above, we are studying predicted functional domains of *DBC2* to understand the biological roles of *DBC2*. Computational analysis revealed that *DBC2* contains

TABLE 1 Networks with high scores (>10)

Networks	Functions	Score	Genes
AATF, ATF2, BRCA1, CBX5, CCNE1, CCNE2, CD44*, CDK2, CHAF1A, CHK*, DDIT3, DHFR*, FBLN1*, FEN1, FN1, FTH1, ID2, IL6, ITGA2, ITGA5, LIG1, MAX, MCM6, MFAP1, PCNA, POLD3, RB1, RBBP7, RPA1, RRM1, TFAP2C, TFDP1 (TRANSCRIPTION FACTOR Dp1)*, UBE2I, UBTF, WEE1	Cell cycle control S phase G_1/S transition Apoptosis Proliferation	47	35
ACTR2, ACTR3, **BET1**, BET1L, CDC42, CDC42EP5, CEP2, **COPB**, **COPG**, GOLGB1, GOSR2, **MSF**, **NEDD5**, **NSF**, PNUTL1, **RAB4A***, RAB5A, RBET1, SEC22A, **SEPT6**, **SEPT8**, SLY1P, **SNAP25***, **STX16**, STX4A, **SYBL1**, **SYPL**, **TAP2**, **TGFBR3**, VAMP2, VDP,VPS45A, WAS, **WASPIP**, **YKT6**	Cytoskeleton Assembly of Golgi Membrane trafficking Fusion	15	18
ARHGEF12, ATM, CASP3, CASP7, **CAV2**, CCND3, **CD59**, **CDC2L2**, CSPG6, EIF2S1, GCLC, **GCLM**, **GNA11**, GNA13, GNAI2, GNAI3, **GNAQ**, GNB1, **GNB5**, GSN, **LGN**, NUMA1, **PCDH7**, **PIK3CA**, PPP1CA, **PPP1R3C**, PRKR, **RAD21***, **RDX**, SMC1L1, SREBF2, SRF, **TARBP2**, **TAX1BP1**, **WEE1**	Cell death Apoptosis Morphology Autophagy	14	17
ACTB, CASK, CASP3, CDH1, CDH2, CTNNB1, **EPB41L1***, FGF1, FGF10, FGF2, FGF7, FGFR1, **FGFR2***, FMR1, **FOSL1**, **FXR2**, GRIK2, GSK3B, **GYS1**, **HERPUD1**, **ITPR1***, JUP, MYO5A, **MYO5C**, **PPP1R2**, PSEN1, RBBP4, SDC1, **SDC2**, **SERPINE1**, **SERPINE2**, **SMARCA3**, **SMARCA4**, **U2AF1**	Apoptosis Differentiation Mitogenesis Growth Proliferation	12	16

Focus genes are shown in bold type. Asterisks indicate genes that belong to multiple networks.

putative protein-protein interaction domains (BTB). We utilized immunoprecipitation and a yeast two-hybrid system to isolate interacting partners. Golgin-84 was identified as one of the candidates. Subcellular localization studies revealed that some *DBC2* proteins migrate around the Golgi apparatus where Golgin-84 resides. Additionally, we found that *DBC2* localization seems not to be affected by Brefeldin A (BFA) which disturbs Golgi structure, whereas BFA causes redistribution of Golgin-84 to cytoplasm, more specifically, the endoplasmic reticulum. This indicates that *DBC2* is not a constituent of the Golgi apparatus itself and that the interaction between *DBC2* and Golgin-84 requires integrity of the Golgi apparatus. Although *DBC2* physically interacts with Golgin-84 in both yeast and mammalian cells, their functional relationship remains unclear.

Transcriptional Regulation of DBC2

M. Hamaguchi [in collaboration with N. Sato, Tokyo Metropolitan Institute of Medical Science]

Transcriptional regulation of *DBC2* was investigated since *DBC2* expression is extinguished in approximately half of breast and lung tumors whereas its expression is detected in other types of cancer. To study transcriptional regulation of *DBC2*, we first determined the precise location of *DBC2* promoters. Upstream genomic sequences of the *DBC2* gene were screened by computational analysis, leading to the isolation of four candidate promoter sequences. They were then tested by a luciferase assay in which DNA fragments with promoter activity turn on a reporter gene (luciferase) and therefore are identified. All predicted promoter sequences demonstrated strong promoter activities in all cells tested (Fig. 1). Since the most common mechanism to suppress promoters is methylation of the promoter region, we are screening cancer cell lines for methylation status of these *DBC2* promoters.

DBC2 and Cell Cycle Control

M. Hamaguchi, V. Siripurapu, C.B. Meyer, C.A. Sorensen

We discovered that *DBC2* negatively regulates the CCND1 protein that is essential for the cell cycle control at G_1/S transition. It also down-regulates CCNE, which is downstream from CCND1. Certain oncogenes involved in breast cancer development such as

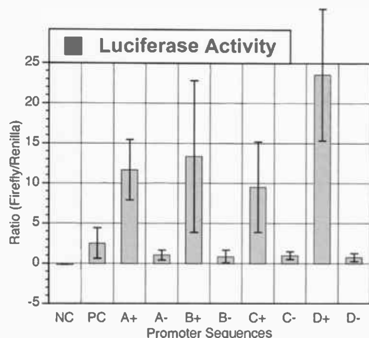

FIGURE 1 Luciferase assay. Four candidate promoter sequences (*A* through *D*) were tested with Dual Glo Luciferse Assay kit and EG&G Berthold Lumat LB9507. NC and PC indicate negative and positive controls provided by the supplier. Plus and minus indicate sense and antisense, respectively. The ordinate represents signal ratios between Firefly and Renilla. All four sense sequences demonstrated very strong promoter activities.

ras/c-*erb-B2* depend entirely on CCND1 to connect to the cell cycle machinery and some do not. If *DBC2* demonstrates anti-tumor activity via CCND1, it is conceivable that cancer cells will display different sensitivity 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? These questions must be answered because they are directly related to the control of tumor growth by *DBC2*.

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Jennifer Meth, lab tech, Masaaki Hamaguchi's lab.

RNA INTERFERENCE: MECHANISMS AND APPLICATIONS

G. Hannon M. Carmell, K. Chang, A. Denli, M. Dus, M. Golding, L. He, J. Liu, E.P. Murchison, Y. Qi, F. Rivas, J. Silva, D. Siolas

Several years ago, work in a free-living nematode, *Caenorhabditis elegans*, uncovered a previously unknown biological response through which an organism exposed to double-stranded RNA specifically silenced genes that share homology with that nucleic acid. This phenomenon, called RNA interference (RNAi), has since been shown to be an evolutionarily conserved pathway, present in organisms ranging from fungi to plants to mammals. We have striven to understand the mechanistic basis of this response using biochemical approaches in several systems, including *Drosophila* and mammalian cells. We have identified numerous components of this pathway, including the key initiating enzyme, Dicer and components of the RISC (RNA-induced silencing complex) effector machinery. Studies this year have begun to unravel how initial processing steps for microRNAs (miRNAs), the endogenous noncoding RNAs that regulate gene expression through the RNAi pathway, are matured. This involves the action of the Microprocessor complex and a newly discovered component of that complex, called Pasha. We have also continued to focus on the inner workings of RISC. Working with Leemor Joshua-Tor here at CSHL, we have identified the key catalytic component of RISC as Argonaute2. Our efforts to understand the biological function of the RNAi machinery in mammals have continued to bear fruit with the creation of a number of animals that lack Argonaute family members. A subset of these mutant animals die early in development. Additionally, as predicted from our biochemical studies, mice lacking Argonaute2 are unable to mount an experimental RNAi response. An analysis of the phenotypic defects in these animals may reveal key aspects of the role of RNAi in normal development.

Exploiting our understanding of RNAi biochemistry, we have developed powerful tools for experimental manipulation of gene expression in mammalian cells and animals. With the help of Dick McCombie here at CSHL, Steve Elledge at Harvard, and colleagues at Merck (CSHL alumnus Michelle Cleary) and OSI (Julie Kan), we have developed a large-scale library of RNAi-inducing vectors that presently covers two thirds of all genes in the human and mouse genomes. We hope to complete the development of these tools next year.

Last year saw a number of changes to the laboratory. Patrick Paddison graduated and accepted a position as a Cold Spring Harbor Fellow. Jian Du accepted a position at Lily, and Kim Scobie started graduate school at Columbia. We were joined by two graduate students: Monica Dus from the Watson School of Biological Science and Sihem Cheloufi from Stony Brook University. Mike Golding and Yijun Qi joined as postdoctoral fellows.

MECHANISMS OF RNAi

Drosha and Pasha: The Microprocessor

A. Denli

RNAi is involved in numerous phenomena in the cells, one of which is the regulation of endogenous genes by host-encoded transcripts, microRNAs. miRNAs' journey to the RNA-induced silencing complex (RISC) starts in the nucleus, where they are transcribed as long RNAs, which are often up to kilobases long. A nuclear RNase III enzyme, Drosha, is responsible for the initial cleavage of these long transcripts into imperfect hairpins called pre-miRNAs. These RNAs move into the cytoplasm via exportin-5, where they are further processed into mature miRNAs by Dicer. These mature miRNAs are incorporated into RISC, which regulates genes by either translational inhibition or mRNA cleavage. To bring this pathway into better focus, I have taken a biochemical approach to identify new components. By using a candidate gene approach supported by biochemical purifications, we identified a Drosha-associated protein, Partner of Drosha (Pasha), and characterized the complex in which they reside, which we named Microprocessor. We have also shown that Pasha is required for miRNA metabolism in flies, humans, and worms. I am currently working on identification of other proteins in the Microprocessor complex.

Argonaute2 Is the Catalytic Engine of RNAi

J. Liu

Double-stranded RNA in most eukaryotic cells triggers a sequence-specific gene silencing response known as RNAi, carried out by the RISC. RISC contains two signature components, small interfering RNAs (siRNAs) and Argonaute family proteins. RISC functions through several distinct mechanisms: mRNA cleavage and degradation, translational repression, and chromatin modification. We recently showed that the multiple Argonaute proteins present in mammals are both biologically and biochemically distinct, with a single mammalian family member, Argonaute2, being responsible for mRNA cleavage activity. A nuclease domain within Argonaute contributes the "Slicer" activity to RISC and provides the catalytic engine for RNAi. I am carrying out further studies of the RISC complex to identify additional components of the effector complex, identify and validate the physiological targets of miRNAs, and dissect the mechanism of gene silencing by miRNAs.

Reconsitution of RISC from Purified Components

F. Rivas

Previously, our laboratory, in collaboration with Dr. Leemor Joshua-Tor here at CSHL, presented genetic, biochemical, and structural studies implicating Argonaute proteins as the catalytic core of the RNAi effector complex, RISC. Site-directed mutagenesis of the putative active site of human Argonaute2, determined in collaboration with Joshua-Tor's laboratory by using structural information from the PIWI domain of an archeal Argonaute, *Pyrococcus furiosus* Ago, inactive Slicer activity strongly suggested that Argonaute2 is the elusive endonuclease in RISC. To definitely demonstrate that this is the case, working with Niraj Tolia in the Joshua-Tor lab, we have now shown that recombinant human Argonaute2, purified from bacteria, can combine with an siRNA to form minimal RISC that accurately cleaves substrate RNAs. Recombinant RISC shows many of the enzymatic properties of RISC purified from human or *Drosophila* cells in terms of K_m, k_{cat}, V_{max}, and Mg^{2+} dependence, but also surprising features. It shows no stimulation by ATP, suggesting that factors promoting product release are missing from the recombinant enzyme. The RISC reconstitution system reveals that the siRNA 5′ phosphate is important for the stability and the fidelity of the complex but is not essential for the creation of an active enzyme. These studies demonstrate that Argonaute proteins catalyze mRNA cleavage within RISC and provide a source of recombinant enzyme for detailed biochemical studies of the RNAi effector complex. Following the observation that the activity of recombinant Ago2 was not augmented by ATP, which is consistent with the existence of an ATP-dependent product release factor in vivo, I have now focused on the identification of this activity, a putative helicase that we have provisionally termed "Twister."

Biochemical Specialization of *Arabidopsis* Dicers

Y. Qi

In *Arabidopsis*, there are four Dicer-like (DCL) enzymes and ten Ago proteins. Although extensive genetics analyses have yielded significant amounts of information on the genetic aspects of RNA silencing in plants, information about biochemical basis of these processes is mostly lacking.

In the past year, we developed protocols to assay *Arabidopsis* Dicer and RISC activities in vitro. We investigated the biochemical properties of two *Arabidopsis* DCLs and found that they have distinct properties. DCL1 produces 21-nucleotide small RNAs, whereas DCL3 produces 24-nucleotide small RNAs. Such different-sized small RNAs have previously been linked to different silencing outcomes through genetic studies and through the use of plant viral inhibitors of silencing. We also showed that Ago1 is the plant "slicer." It is the first foray into the biochemical dissection of RNAi pathways in *Arabidopsis* and provides a foundation for exploiting the rich genetic resources that have been developed in this system for understanding the intricacies of RNA silencing in plants.

Role of Vig and PIWI Proteins in RNAi

M. Dus

Our lab recently showed that Argonaute proteins are not only one of the hallmarks of RNAi, but also the very catalytic core of this process. In almost all organ-

isms where RNAi has been studied, this family of proteins is further subdivided into members that present closer homology with either *Drosophila* PIWI or AGO1. Although our understanding of the "AGOs" is rapidly developing, knowledge of the functions of the PIWI family members is still lacking. The aim of my research is to uncover the biochemical functions of the PIWI subfamily using flies and mice as model systems. In particular, I will test the involvement of these proteins in transposon regulation and heterochromatin. Furthermore, I am trying to dissect the cytoplasmic and nuclear functions of Vasa intronic gene (VIG), especially focusing on its role in heterochromatin formation.

BIOLOGICAL FUNCTIONS OF THE RNAi MACHINERY

Function of Argonaute Family Members in Mammalian Development

M. Carmell

Argonaute family members have been shown to be essential for RNAi/PTGS (posttranscriptional gene silencing) in several organisms, including *Neurospora* (QDE-2), *Arabidopsis* (AGO1), and *C. elegans* (*rde-1*). Argonautes are core components of RISC complexes and function both in mRNA destruction and in translational inhibition. Interestingly, Argonaute genes have also been implicated in control of development in several organisms. It is unknown what roles of the RISC complex are mediated by Argonaute family members, and whether developmental phenotypes result from loss of discrete regulatory functions or from a more general misregulation of silencing mechanisms. To address the role of Argonautes in the mammalian system, conventional mouse knockouts of the Argonaute gene family are under way. This year, we have shown that the multiple Argonaute proteins present in mammals are both biologically and biochemically distinct, with a single mammalian family member, Argonaute2, being responsible for mRNA cleavage activity. This protein is essential for mouse development, and cells lacking Argonaute2 are unable to mount an experimental response to siRNAs. Analyses of other Argonaute knockouts, including Ago3 and Miwi2, reveal other discrete biological roles, with Ago3 being nonessential and Miwi2 being important for spermatogenesis.

Genetic Studies of RNAi in Mouse Embryonic Stem Cells

E.P. Murchison

In mouse, Dicer is encoded by a single locus whose protein product is required for the production of small RNAs involved in RNAi and related phenomena. To further characterize the role of RNAi in genome regulation in mammals, we created conditional Dicer mouse embryonic stem (ES) cells in which the only remaining copy of Dicer can be deleted by expression and activity of Cre recombinase.

We find that upon loss of Dicer, ES cells fail to proliferate, revealing a requirement for Dicer activity and Dicer products in the sustained growth of these cells. As expected, ES cells lacking Dicer are unable to carry out posttranscriptional gene silencing in response to double-stranded RNA and cannot produce mature miRNAs. Furthermore, Dicer-deficient ES cells accumulate centromere-derived transcripts, analogous to the situation seen in *Schizosaccharomyces pombe*, but retain centromere methylation. We are currently characterizing these phenotypes with the hope of uncovering the underlying cause of the proliferation phenotype. In addition, we are using the conditional ES cells to generate Dicer conditional mice.

An miRNA as a Potential Human Oncogene

L. He

I have been examining potential roles of miRNAs in tumor formation. To date, more than 200 miRNAs have been described in humans. Although there is circumstantial evidence suggesting that these regulatory, noncoding RNAs have a role in tumor development, it still remains largely obscure whether a micron can function as an oncogene. Work of a former postdoc, Scott Hammond, led us to focus our efforts on a particular miRNA cluster, miR17-92. Working with new faculty member Scott Powers, we compared B-cell lymphoma samples and cell lines to normal tissues and found 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

the 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, an experiments carried out with Scott Lowe's lab here at CSHL. Tumors derived from hematopoietic stem cells (HSCs) expressing *mir17-92* and c-*myc* are distinguished by an absence of the apoptosis that is 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.

APPLICATION OF RNAi AS A GENETIC TOOL IN MAMMALS

Synthetic shRNAs as Highly Potent RNAi Triggers

D. Siolas

Designing highly potent silencing triggers is key to successful application of RNAi in mammals. Recent studies suggested that assembly of RNAi effector complexes (RISC) is coupled to Dicer cleavage. I therefore examined whether transfection of optimized Dicer substrates might give an improved RNAi response. Dicer cleavage of chemically synthesized short hairpin RNAs (shRNAs) with 29-bp stems and 2-nucleotide 3′ overhangs produced predictable homogeneous small RNAs comprising the 22 bases at the 3′ end of the stem. Consequently, direct comparisons of synthetic siRNAs and shRNAs that yield the same small RNA became possible. Synthetic, 29-mer shRNAs were found to be more potent inducers of RNAi than siRNAs. Maximal inhibition of target genes can be achieved at lower concentrations, and silencing at 24 hours is often greater. These studies provide an improved methodology for experimental silencing in mammalian cells using highly potent RNAi triggers. This remains a critical issue both for cell culture studies and for potential therapeutic use in vivo. Mapping the predominant 22-nucleotide sequence that appears in RISC from each of these shRNAs now permits the combination of this more effective triggering method with rules for effective siRNA design.

Using RNAi to Understand Epigenetic Control of Gene Expression

M. Golding

Since arriving in May, my research has focused on applying the shRNA library to studies of epigenetic gene silencing. Here, I hope to conduct several screens that will further our understanding of the genetic factors involved in the process of gene silencing. Several model systems are being used to study the processes of epigenetic gene silencing as they relate to tumor suppressor gene silencing, double-stranded RNA-directed DNA methylation, and genomic imprinting.

The first phase of my project sought to determine which promoter elements were best suited to driving the new mir30-based shRNA expression constructs. Several different vectors were built to test which promoter types were most effective both in transient transfection and in stable integration. Concurrent with studies of the different promoter elements, assays to measure the expression of several candidate genes were developed using TaqMan real-time polymerase chain reaction (PCR). shRNA constructs targeting these genes were placed in the vector system described and the mRNA expression levels of their targets were monitored by real-time RT-PCR. This created a reliable system with which to quantitatively monitor the efficacy of the different vector designs.

The second phase of my studies has sought to find a reliable system in which to study epigenetic gene silencing. Initial trials have begun to look at tumor suppressor gene silencing and the potential synergistic effects of the shRNA library with a new class of drugs termed HDAC inhibitors. Trials using the drug SAHA have begun. Concurrent with these studies, we are collaborating with Colin Stewart (National Institutes of Health) using the shRNA library in studies of genomic imprinting.

Using RNAi to Discover Targets for Combination Drug Therapy

J. Silva

RNAi suppression studies are one of the most informative approaches to identify gene function. Using the shRNA library that we have previously developed, I am performing loss-of-function screens to uncover

genes involved in the acquisition of tumor features. I am interested in two different processes: modulation of the p53 response (activation or attenuation upon a stress stimulus) and anchorage-independent growth. Additionally, I am conducting a screen to discover new genes that enhance the response to anti-EGF (epidermal growth factor) receptor therapy (Tarceva). Finally, in collaboration with Despina Siolas, I am exploring the possibility of using RNAi technology to reveal synthetic-lethal interactions with the most commonly alterated genes in cancer (*p53*, *ras*, *Rb*, *p16*).

Microarray-based Methods for Analyzing Genetic Screens in Mammals

K. Chang

DNA microarray analysis has come a long way since its introduction by Patrick Brown (Stanford University, Palo Alto, California) and colleagues in the early 1990s. In recent years, advancements in the area of chip fabrication have paved the way for the development of high-density oligonucleotide microarrays. Its applications have revolutionized the field of genomics. During the past year, we have been developing the use of such microarrays in loss-of-function RNAi screens in mammalian cells. The creation of our RNAi library has made it possible, for the first time, to perform synthetic-lethal analyses in animal cells in ways similar to those that have made yeast such an amenable model organism. In our approach, stable populations expressing pools of shRNAs are made by transducing library cassettes into target cells. Upon selection, cells depleted or enriched from a population, following treatment (e.g., drug, siRNA knockdown, or left untreated), can be tracked using "DNA bar codes" (24 mers or 60 mers fabricated on DNA microarrays) associated with specific shRNAs such that any gain or loss in copy number is a direct consequence of the expressed shRNA on the cell. Using this approach, we have been successful monitoring copy changes reproducibly in mouse and human cells using defined pool complexities. In addition, we have been testing other methods for tracking cells using "RNA bar codes," which can also be used to prevalidate the effectiveness of our RNAi library.

We are currently applying this paradigm in synthetic-lethal screens to elucidate drug action (e.g., Tarveva, Velcade, SAHA, and Rapamycin) and to gain new insight into the biology of genetic diseases. This is an exciting time as this will be a promising new approach for drug discovery and tailoring therapies for cancer and other genetic diseases, and a new tool for functional genomics.

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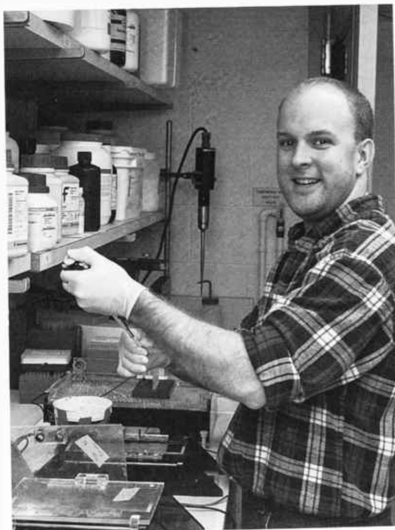

Michael Golding, postdoc, Greg Hannon's lab.

SPORADIC HUMAN GENETIC DISEASE

E. Hatchwell B. Gildor T. Lee
C. Hubley I. Mileva
S. Kantarci J. Roohi

We are interested in the application of modern 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 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., velocardiofacial syndrome [VCFS] which is associated with heterozygous deletions of 3 Mb).

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, cDNA (PCR product), and large clone (based on bacterial artificial chromosomes [BACs]) DNA microarrays. We have generated BAC microarrays over the last 3 years, at increasing density, and our current arrays contain a complete tile of the human genome (19,000 BACs), 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
- Congenital heart disease
- Complex phenotypes and de novo, apparently, balanced translocations
- Development of methods to validate putative aCGH variations
- Development of methods to generate haploidy from diploidy, in order to more effectively analyze heterozygous changes
- Genome-wide array-based analysis of DNA methylation, with particular reference to monozygotic (MZ) twins.

We were sorry to have had to say goodbye last year to Dr. Sibel Kantarci, a postdoc who was with us for almost 3 years, and who is currently pursuing a career in clinical molecular genetics at Massachusetts General Hospital, Boston. In addition, our valued Level III Technician, Cindy Lee, who was instrumental in setting up our BAC aCGH technology, left at the start of 2004, to pursue a career as an embryologist at New York University. We were pleased to welcome Izolda Mileva, an experienced technician from the Greenwood Genetic Center in South Carolina, and Jasmin Roohi, an M.D. Ph.D. student from Stony Brook University, who is our first Cody Center Fellow.

Autism Spectrum Disorder

J. Roohi

We are collaborating with workers at the Cody Center, Stony Brook, in an interdisciplinary study of a cohort of patients diagnosed with autism spectrum disorder (ASD). In addition to detailed diagnostic interviews/questionnaires by experts on 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 Fragile-X type A (FRAXA). To date, approximately 80 patients have been assessed. What has become very clear already is how heterogeneous this disorder is—fully 50% of patients were dysmorphic or had 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 (FRAXA, Angelman syndrome, Rett syndrome, etc.). 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, in the absence of a parallel clinical/phenotypic analysis, is unlikely to yield answers on its own.

In addition to our medical genetics examinations, a selected group of patients are to undergo detailed imaging analysis, in collaboration with Dr. Benveniste and colleagues at Brookhaven National Laboratory. We have been applying aCGH using BAC arrays to our cohort of ASD patients and have already detected many genomic copy-number abnormalities. The challenge is to (1) validate these changes and (2) estimate whether any validated changes represent population variants or are specific to ASD.

See below for some of our approaches to addressing both challenges.

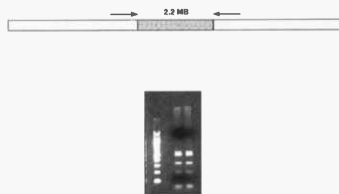

FIGURE 1 PCR of the "junction fragment" generated by a de novo 2.2 Mb microdeletion in a patient with developmental delay reveals at least four distinct bands, suggesting that the deletion is mosaic at the molecular level.

Congenital Heart Disease

I. Mileva

We have a long-standing interest in the genetics of congenital heart disease (CHD); 1% of all 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 approximately 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 ~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 recently secured funding from Harrison's Heart Foundation, a private charity devoted to funding research into this understudied area. We have active collaborations with two major centers of excellence, from whom we obtain DNA samples on well-characterized individuals with CHD: Dr. Piers Daubeney at The Brompton Hospital, London, and Dr. Steve Webber at The Children's Hospital, Pittsburgh. To date, we have obtained samples from 150 patients with CHD and are planning to study these with our new tiling path BAC arrays during the course of this next year.

Complex Phenotypes and De Novo Translocations

S. Kantarci, I. Mileva, B. Gildor

We collaborate actively with Dr. Helga Toriello (Spectrum Health, Grand Rapids, Michigan), a distinguished dysmorphologist, who supplies us with samples from chromosomally normal individuals with complex disorders. In one such individual, we have uncovered a de novo 2q31 deletion that we have studied in detail and demonstrated to be 2.2 Mb in size. Analysis of the breakpoint regions in this patient has revealed that the deletion is mosaic at the molecular level, there being four distinct junction fragments present in DNA isolated from peripheral blood. This is the first such example of which we are aware (see Fig. 1).

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. Studies are under way to search for mutations in this gene in Joubert Syndrome patients who are chromosomally normal.

Validating aCGH-detected Changes/Determining Population Frequencies

J. Roohi

There are two major problems to consider:

- Validating changes suggested by aCGH.
- Determining whether validated changes are specific to the phenotype being investigated or whether they represent variants in the normal population.

Recent work from a number of groups suggests that most normal individuals harbor at least a dozen copy-number changes, when compared to the consensus human genome sequence. Clues to this reality

have been present historically, including our own discovery 3 years ago of a heterozygous microdeletion, 182 kb in size, which we demonstrated to be present in 5% of all normal individuals. It is imperative that the new technologies be used to study a large number of normals to determine which genomic copy-number alterations are population variants and, if so, at what frequency they occur in different populations. Such studies were conducted in the 1960s when chromosome analysis was performed on 10,000 unselected newborns, in order to determine the frequencies of various aneuploidies.

Although FISH (fluorescent in situ hybridization) remains the mainstay of validating changes suspected on aCGH, this method is neither easily scaleable nor always possible, as it relies on the availability of living cells. Molecular methods for validating such changes include the use of polymorphic markers, but this method suffers from a general lack of knowledge of population heterozygosity and the possibility of lack of informativeness in a given individual.

We have recently attempted to use real-time (quantitative) polymerase chain reaction (PCR) to validate heterozygous deletions and have had significant successes. Our aim is to continue to optimize qPCR for this purpose, in order to screen large numbers of individuals with the relevant phenotype and an equal number of controls. We are also developing methodologies that will allow the simultaneous interrogation of thousands of individuals to determine their status for a given copy-number alteration.

Development of Methods to Generate Haploidy from Diploidy

C. Hubley

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 the germ line) 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, with Particular Reference to Monozygotic Twins

E. Hatchwell

The underlying causes of phenotypic variation in human genetic disorders are still largely not understood. "Genetic background" is often invoked to explain variation in nonidentical individuals, but 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 in the past to the examination of specific genes.

We have embarked on the design and optimization of 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. Our array design is now ready and we shall study sets of MZ twins, both normal and discordant, for a given phenotype (reading ability; a collaboration with Ian Craig, Institute of Psychiatry, London).

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THE ORIGIN AND VIABILITY OF CANCER CELLS

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

CELL FUSION AS A CAUSE OF CANCER AND ITS VARIABILITY

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 cell fusion 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 cells 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, such as 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. Survival and proliferation of fused cells, most of which die or arrest in cell cycle, depend on many factors that are largely unclear and appear to include cell type and proliferation status of the parental cells. Given these constraints, the question is whether virus-induced cell fusion can result in proliferating cells that have the potential to become cancerous.

While investigating why a cell line is fusogenic, we found that the cells released exosomes that contained the Mason-Pfizer monkey virus (MPMV), a pathogen that is found in primates, including humans. The fusion occurred shortly after exposure to the virus and was unaffected by AZT, an inhibitor of reverse transcriptase, which excluded the requirement of the virus integration. However, the fusion was also prevented by γ-irradiation, which implied a requirement for an intact viral genome. To reconcile these observations, we proposed a model that interaction of MPMV with cells induces release of an activity, which we named ϕ, that fuses uninfected cells. We provided evidence in support of this model.

Because proliferation is an absolute requirement for oncogenic transformation, we tested whether cells fused by ϕ proliferated. We found that cells destined to die could instead produce transformed proliferating progeny if fused by ϕ. In particular, we found that neighboring cells that are destined to die could instead produce transformed progeny if the cells are fused by a virus that happened to be nearby.

Overall, we proposed how a retrovirus can generate proliferating abnormal cells without becoming part of them. A current view is that retroviruses 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 INDUCED BY ONCOGENES AS A BASIS FOR KILLING CANCER CELLS

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

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

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 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 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 determined the pathway of apoptosis involved and began to analyze how a depletion of glutamine can activate this pathway. We evaluated several possibilities suggested by previous studies, such as interference with energy metabolism or protein synthesis, and found that none of them can explain our observations. We are currently investigating other options.

Overall, 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

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E. de Stanchina M. Narita M. Spector J. Zilfou
R. Dickins

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 and Tumor Suppressor Genes

A. Bric, D. Burgess, E. Cepero, E. de Stanchina, R. Dickins, M. Hemann, M. McCurrach, L. Zender, J. Zilfou [in collaboration with G. Hannon and W. Tansey, Cold Spring Harbor Laboratory; D. Livingston, Dana Farber Cancer Center; and former laboratory member A. Samuelson]

Normal cells possess intrinsic tumor suppressor mechanisms that limit the consequences of aberrant proliferation (for review, see Lowe et al. 2004). 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 in part via the p53 tumor suppressor pathway and limits tumor development. We have previously shown that oncogenes can engage the ARF-p53 pathway to promote apoptosis and that disruption of this pathway cooperates with oncogenes to promote oncogenic transformation in vitro and tumor development in vivo. We are currently interested in identifying additional components of these programs and in understanding how they function in a "tumor suppressor network."

The adenovirus E1A oncoprotein promotes proliferation and transformation by binding cellular proteins, including members of the Rb protein family, the p300/CBP transcriptional coactivators, and the p400/TRRAP chromatin-remodeling complex. E1A also promotes apoptosis, in part, by engaging the ARF-p53 tumor suppressor pathway described above. This year, we completed a study showing that E1A induces ARF, 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, whereas E1A inactivates Rb, it requires p400 to efficiently promote cell death. These results identify p400 as a regulator of the ARF-p53 pathway and a component of the cellular machinery that couples proliferation to cell death.

Like E1A, the cellular c-Myc oncoprotein drives proliferation and cell death. This year, we collaborated with William Tansey to characterize the role of a highly conserved, but little studied, element within the central region of c-Myc, termed "Myc box III" (MbIII). We show that MbIII is important for transcriptional repression by Myc and for transformation both in vitro and in a mouse model of lymphomagenesis (Herbst et al. 2005). Curiously, disruption of MbIII decreases transformation activity by increasing the efficiency with which Myc can induce apoptosis, suggesting that MbIII is a negative regulator of programmed cell death. These findings reveal a role for MbIII in Myc

functions and establish that the oncogenic capacity of Myc is linked directly to its ability to temper the apoptotic response. We are continuing to study Myc activity in vivo and, in particular, are studying the oncogenic properties of naturally occurring *myc* point mutants found in Burkitt's lymphoma.

An important aspect of our research in apoptosis continues to explore the biological roles of p53 in tumor suppression. p53 regulates diverse antiproliferative processes such that cells acquiring *p53* mutations have impaired cell cycle checkpoints, senescence, apoptosis, and genomic stability. This year, we used stable RNA interference (RNAi) to examine the role of *PUMA*, a p53 target gene and pro-apoptotic member of the Bcl2 family, in p53-mediated tumor suppression. *PUMA* short hairpin RNAs (shRNAs) efficiently suppressed PUMA expression and p53-dependent apoptosis but did not impair nonapoptotic functions of p53 (Hemann et al. 2004). Like *p53* shRNAs, *PUMA* shRNAs promoted oncogenic transformation of primary murine fibroblasts by the *E1A/ras* oncogene combination and dramatically accelerated *myc*-induced lymphomagenesis without disrupting p53-dependent cell cycle arrest. However, the ability of PUMA to execute p53 tumor suppressor functions was variable because, in contrast to *p53* shRNAs, *PUMA* shRNAs were unable to cooperate with oncogenic *ras* in transformation. These results demonstrate that the p53 effector functions involved in tumor suppression are context-dependent and, in some settings, depend heavily on the expression of a single effector. Additionally, they demonstrate the utility of RNAi for evaluating putative tumor suppressor genes in vivo.

Control of Cellular Senescence

D. Burgess, E. de Stanchina, M. Narita, M. Narita, S. Nuñez, J. Zilfou [in collaboration with G. Hannon, Cold Spring Harbor Laboratory; and David Livingston, Dana Farber Cancer Institute; also involving former laboratory members E. Querido and G. Ferbeyre]

Cellular senescence was originally described as the process that accompanies replicative exhaustion in cultured human fibroblasts, and it is characterized by a series of poorly understood markers. Senescent cells remain metabolically active but 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 (Lowe et al. 2004), and suboptimal cell culture conditions. These observations have led us to propose that senescence acts in parallel with apoptosis as a cellular response to stress. Indeed, based on this analogy, much of our work on senescence is guided by our past experience on apoptosis.

We continue to identify and characterize new factors that modulate senescence. For example, we previously showed that the promyelocytic leukemia (PML) gene is up-regulated during senescence and now have extended these observations by showing that *PML* is a p53 target gene that contributes to both senescence and apoptosis (de Stanchina et al. 2004). Our results identify a new element of PML regulation and establish PML as a mediator of p53 tumor suppressor functions. Recent work indicates that the role of PML in mediating p53 responses may extend to many tissues and cell types. We also worked with David Livingston to characterize the role of the p400 protein in regulating cellular senescence. This study showed that p400 is a component of the p53 cell-cycle-arrest pathway, having a key role in regulating the transcription of p21 (a cyclin-dependent kinases inhibitor). Notably, acute depletion of p400 expression using RNAi led to premature senescence of untransformed human fibroblasts in a p53/p21-dependent manner (Chan et al. 2004). Taken together with our studies on apoptosis, these data imply that p400 acts as a central player in cellular decisions that couple proliferation to cell death and senescence.

We are also interested in identifying components of 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, SAHF) that is dependent on the Rb tumor suppressor and is associated with the stable silencing of E2F target genes (see comment in Narita and Lowe 2004). To gain insights into the chromatin changes linked with senescence, we have begun a proteomics analysis to identify molecules that selectively associate with chromatin in senescent cells. As we identify new chromatin-associated proteins, we will progress toward in vivo studies, where we hope to deconstruct the process using forward and reverse genetic approaches. Here,

our interactions with the Hannon lab on stable RNAi technology are crucial for our future plans.

Evolution of Genomic Instability in Human Tumors

M. Hemann, J. Zilfou [in collaboration with E. Hernando, C. Cordon-Cardo, W. Gerald, and R. Benezra, Memorial Sloan-Kettering Cancer Center; and former laboratory member Z. Nahle]

Aneuploidy is a hallmark of advanced human cancers, yet the molecular defects underlying this trait, and whether they are a cause or a consequence of the malignant phenotype, are not clear. For several years, we have studied the role of p53 in controlling chromosomal stability, and the relevance of this function for p53 tumor suppressor activities. This year, we began to study the role of the Rb protein in controlling genomic integrity (Hernando et al. 2004). Notably, mutations that disable the Rb pathway are also common in human cancers. These mutations promote tumor development, in part, by deregulating the E2F family of transcription factors, leading to uncontrolled cell cycle progression. We showed that the mitotic checkpoint protein Mad2 is a direct transcriptional target of E2F and, as a consequence, is aberrantly overexpressed in cells with defects in the Rb pathway. Concordantly, Mad2 is overexpressed in several tumor types, where it correlates with high E2F activity and poor patient prognosis. Generation of Rb pathway lesions in normal and transformed cells produces aberrant Mad2 expression and mitotic defects leading to aneuploidy. Remarkably, Rb inactivation *requires* Mad2 up-regulation to fully produce ploidy alterations. Our results reveal how chromosome instability can arise as a byproduct of defects in cell cycle control that compromise the accuracy of mitosis and identify a new mechanism of genomic instability that may contribute to the high frequency of aneuploidy observed in advanced human cancers.

Molecular Genetics of Drug Sensitivity and Resistance

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

A major goal of our research is to understand the biological and molecular basis of drug sensitivity and resistance in tumors. 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 *E*μ-*myc* transgenic mouse, which develops B-cell lymphomas at short latency with high penetrance. Using this system, we have identified a number of biologic and genetic determinants of treatment sensitivity in vivo (for review, see Lowe et al. 2004).

This year, we examined how constitutive signaling by Akt compared to Bcl-2 overexpression in promoting oncogenesis and drug resistance in vivo (Wendel et al. 2004). We showed that Akt, like Bcl-2, cooperates with Myc during lymphomagenesis, in part by evading p53 action, leading to highly disseminated lymphomas that are pathologically indistinguishable. We also showed that the mTOR inhibitor rapamycin (which interferes with one of several pathways downstream from Akt) synergizes with conventional therapy in lymphomas expressing Akt but not those expressing Bcl-2. Interestingly, enforced expression of eIF4E, which acts downstream from mTOR to regulate translation initiation, recapitulated Akt's ability to promote oncogenesis and drug resistance, although eIF4E-expressing lymphomas were refractory to rapamycin.

These results have important ramifications for understanding Akt action in oncogenesis and drug resistance and for considering the use of targeted therapeutics in the clinic (see commentary by McCormick, *Nature 428:* 267 [2004]). First, our study provided the first demonstration that Akt and eIF4E could promote drug resistance in vivo, and thus implied that translational regulation via eIF4E can be important for Akt-mediated survival signaling. Second, the similarities between Akt an Bcl-2 (a purely anti-apoptotic protein) provided a formal demonstration that, despite the diverse impact Akt signaling has on cell physiology, its anti-apoptotic functions are crucial for oncogenesis. Third, our observation that Akt-mediated chemoresistance (but not that produced by Bcl-2 and eIF4E) could be reversed by rapamycin showed for the first time that small molecules capable of reversing apoptotic defects can restore drug sensitivity in tumors. However, they also underscored the importance of tailoring cancer therapy based on tumor genotype.

We continue to study how Akt signaling influences tumor development, drug resistance, and rapamycin sensitivity and are testing a number of novel "target-

ed" therapies in the *E*μ-*myc* model in order to determine whether any can reverse or circumvent the drug resistance produced by p53 loss. Finally, we began a multi-institutional effort to use mouse AML (acute myelogenous leukemia) models to uncover drug resistance mechanisms and to evaluate new therapies, with the goal of translating this information to human patients. We are excited about the potential of the 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. Finally, we have developed a new mouse model of hepatocellular carcinoma that should allow us to extend our analyses to epithelial malignancies.

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GENOMIC MICROARRAY ANALYSIS OF CANCER

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T. Auletta S. El-ftesi
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This work was carried out in collaboration with M. Wigler, J. Healy, and A. Reiner here at CSHL. 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 get 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 begun surveying the genome of several cancer types to identify regions that have undergone increased or decreased gene copy number, namely, amplifications or deletions, since these changes can be used as markers for the location of oncogenes or tumor suppressor genes, respectively. Regions are being informatically searched for gene candidates and then functional analysis of these candidates is being carried out to determine which genes have oncogenic potential. Once identified, these 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 primarily in 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 cancer, the survival rate for pancreatic cancer is one of the lowest. Treatments can extend survival or alleviate pain but seldom cure the patient. In fact, the mean survival time is approximately 6 months. Because lifespan after diagnosis is very short, the number of patients that receive tumor resections is very low. This translates to few samples available for analysis. Because of this, we are collaborating with many clinicians to put together a useful tumor bank of pancreatic tissue for analysis by ROMA. Currently, we are collaborating with Dr. Ralph Hruban of the Johns Hopkins University School of Medicine, Dr. Daniel Von Hoff of the Arizona Cancer Center, Dr. Vijay Yajnic of Mass General, and Dr. Murray Abrams of Memorial Sloan-Kettering Cancer Center who will be providing pancreatic specimens and invaluable clinical information and expertise. To date, we have analyzed approximately 50 samples and have confirmed that many of the known mutated regions are altered in this set; for example, the INK4a-ARF locus, DPC4, p53, and c-*myc* are all lesions commonly identified in this type of tumor. In addition, there are other less-characterized regions deleted (2p23, 3p, 8p23, 10p15, and 12p) and amplified (1q12, 1q42-43, 2q32, 3q26, 8q, 12q, and 19q) frequently in this cancer, and we are continuing to study these regions to identify possible gene candidate tumor suppressors and oncogenes.

We also have two ongoing projects utilizing ROMA to study other aspects of pancreatic cancer. The first is the study of changes that have occurred in pancreatic metastasis. One of our collaborators, Dr. Christine Iacabuzzo, has developed a unique program

within Johns Hopkins Medical Center—a rapid autopsy program whereby cancer tissue specimens are rapidly resected from patients postmortem. These samples are then transferred to mice to grow as xenografts. We have analyzed seven primary tumors and from two to four metastases for each tumor. The metastases are from liver, lung, lymph node, etc. We have found that the metastases have almost identical copy-number patterns. However, there are several regions that are different among the samples of primary to metastases. The samples of primary that we are analyzing are most likely a heterogeneous population of cells that made up the original primary tumor. Interestingly, it appears that there are also regions that are altered more in the metastases than in the primary, possibly suggesting that the metastases had this region altered and were selected as the metastases. We have identified gene candidates within these regions and are attempting to functionally perform characterization assays to determine the role they have in metastasis.

A second project is an attempt to identify if there are possible copy-number polymorphisms (CNPs), small changes in the genome found in apparently normal people, which correlate with the genetic susceptibility of pancreatic cancer. Johns Hopkins Medical Center has a bank of DNA from normal individuals who have a higher risk of developing pancreatic cancer. This increased risk is based on the fact that these individuals have more than one family member who has developed pancreatic cancer, a normally rare cancer. The DNA samples from these patients are analyzed by ROMA and the CNPs are identified. These are then compared to data of CNPs found in a normal population to remove all those that are found commonly in a set of patients who do not have an increased risk of pancreatic cancer. We have found one interesting CNP that is deleted in one family with increased risk of pancreatic cancer as compared to the reference population. This deletion is very close to the INK/ARF locus, but does not delete any genes within this locus. More specifically, a cluster of interferon genes are deleted. Our clinical collaborators have seen homozygous deletion of this region without deletion of INK/ARF in some patients with pancreatic cancer. We are continuing to characterize this region with the help of our clinical collaborator Dr. Ralph Hruban.

OVARIAN CANCER

We are also focusing on the analysis of ovarian cancer using ROMA. Ovarian cancer has a relatively high incidence and an approximately 50% survival rate. In many patients, the cancer is diagnosed late, often having metastasized, with the first symptom being an accumulation of fluid in the abdominal cavity. There have been few genes discovered that are involved in the progression of ovarian cancer. We will be collaborating with Dr. Michael Pearl of Stony Brook Univerrsity 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 as described below.

We have also analyzed a set of ovarian tumors obtained from Dr. Anne-Lise Borresen-Dale from the Norwegian Radium Hospital. This tumor set has already been analyzed by Dr. Patrick Brown and colleagues for expression analysis. This analysis was able to separate the tumors into two distinct sets that correlated with the clinical findings of survival. We have recently finished the analysis of these tumors and are now informatically analyzing the data to determine if the copy-number fluctuations can be also used to identify the same two groups of patients.

Mouse Cancer Analysis by ROMA

T. Auletta, S. Chen, A. Brady [in collaboration with S. Lowe, Cold Spring Harbor Laboratory; S. Powers, Tularik, Inc; S. Orsulik, Massachusetts General Hospital]

We have developed a mouse ROMA array and have begun analysis of mouse copy number differences in the model systems and liver cancer in collaboration with Dr. Scott Lowe. We have performed mouse ROMA on ten liver cancer samples and have identified several alterations. Three of the tumors have an amplification of a region of chromosome 9 near the centromere. In collaboration with Dr. Scott Powers, the human syntenic region of the mouse chromosome was searched for amplification within a ROMA data

set of approximately 50 primary human tumor samples. This region was also found to be amplified within the human data set and the genes within this region are now being functionally characterized by Dr. Scott Lowe's group to determine if one gene has oncogenic activity.

Since this model system for analysis of cancer has worked well, we have begun a similar project for ovarian cancer. We already have 75 primary tumor samples analyzed and will be analyzing another 30 shortly. We have initiated a collaboration with Dr. Sandra Orsulik who has developed a mouse model of ovarian cancer. We have analyzed ten mouse ovarian cancer samples, comparing the data from mouse tumors with those of human tumors, and we are currently looking for common syntenic regions that are mutated.

Gene Candidate Characterization

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

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

We are currently focusing on two regions of amplification, one present in ovarian cancer and the other in pancreatic cancer. The region found in ovarian is found to be amplified in approximately 80% of tumors that are aneuploid. Generally, these are the most aggressive tumors clinically. Within the data set that we are studying, the amplifications vary widely in dimension and amplitude. Most often, the amplification is very large and uninformative from a positional cloning standpoint. However, one specific tumor had amplified a relatively small region of 3Mb and an amplitude of approximately tenfold; 17 gene candidates from this region were identified. This is a large number of candidates to study for functional characterization and validation. We have begun first steps in developing high-throughput genomic approaches to the functional characterization of the gene candidates within the regions studied by using RNA interference (RNAi) studies utilizing the short hairpin RNA (shRNA) library constructed in Dr. Hannon's lab here at CSHL. Cell lines having this region amplified will be infected with shRNA constructs and growth assays will be performed. This approach is also being taken with a region frequently amplified in pancreatic cancer. One advantage that we have for this region is a cell line that was produced from the very tumor that had a focal amplification of this region, by Dr. Chris Iacobuzio of the Johns Hopkins University School of Medicine. This tumor cell line will be a valuable tool for gene validation studies for this region.

Methylation-specific Oligonucleotide Microarray Analysis

O. Dovirak, S. Chen, A. Brady, J. Healy

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 as well as the sequencing of the genome have made detection of methylation at loci more reliable and accurate. However, few methods can identify methylation changes over the entire genome. We have adapted ROMA to methylation detection oligonucleotide microarray analysis (MOMA). Currently, we are utilizing this to survey the changes that occur in cancer, but in principle, this method would have applications for identifying methylation differences involved in imprinting or other syndromes 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 ($N_{40\text{-}3000}$) (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 have analyzed several cell lines and tumors for methylation changes, and have made several interesting observations. First, reports of global hypomethylation in cancer appear to be correct. We have compared the methylation pattern of tumor samples to several normal tissue samples, either a heterogeneous population of skin or embryonic fibroblasts. We observe large pockets of hypomethylation in the tumor in comparison to the normal. In contrast, there is only sporadic hypermethylation of the tumor genome. The one type of region we recurrently observe as hypermethylated in cancer are regions of genomic amplification. We have further investigated this by amplifying the *DHFR* locus in response to methotrexate. Initially, the response of cells is to overexpress the *DHFR* gene, but with increased amounts of methotrexate, the cells genomically amplify the region. Once the region is genomically amplified, the regions becomes hypermethylated. However, the hypermethylation does not affect the expression level of the genes within the amplified region. Apparently there is a difference between methylation of total genome CpGs and islands of CpGs. Interestingly, in other cell lines and tumors that have amplifications, only with high-level amplifications do we see corresponding hypermethylation of the genomic region. High-level amplification is often amplified in the cell as double minutes (DMs), regions of the genome that are excised and form a circular piece of DNA. If these DMs have origin of replications, they autonomously replicate sorting randomly during cytokinesis. We are currently testing whether hypermethylation only occurs in amplified regions that are DMs or if hypermethylation occurs in high-level amplifications within the chromosome, usually termed HSRs, or homogeneously staining regions. In the future, we will be utilizing the tools we have developed to tease out the members of the methylation pathway that are responsible for this phenomenon.

We are continuing to develop this method. We have redesigned the array to represent known CpG islands as well as CG-rich regions of the genome, to make an array that is more specific for methylation detection and transcription control. It is becoming clear that methylation silencing has an important role in cancer, and we intend to marry both the ROMA and MOMA techniques. 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 obtain a more complete picture of the cancer cell. Ultimately, all of these data will be incorporated together and analyzed alongside the clinical information to determine if any genetic or epigenetic factors correlate to clinical outcome.

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MAMMALIAN FUNCTIONAL GENOMICS

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Our laboratory generates novel mouse models for characterizing mammalian gene function in vivo. By investigating normal developmental processes, we are able to better understand molecular mechanisms that contribute to human diseases such as cancer. The link between mammalian development and cancer is at the heart of understanding how to design more effective cancer therapies that do not have adverse side effects.

We are able to tailor-make mouse strains in an effort to model specific human diseases. For example, we can add or remove genes, we can "cut, copy, and paste" specific regions of a chromosome, and we can even make subtle changes within a chosen gene. This ability to manipulate the mouse genome makes it possible to address a diverse array of biological questions to investigate gene function. In addition, these mouse models are invaluable for understanding pathogenesis and for designing novel treatments for human disease.

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

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

A. Bagchi, Y. Wu

Goals: To generate mouse models that mimic human cancer syndromes and to functionally annotate genes mapping to human chromosome 1p.

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

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

Within the past year, we have begun to develop novel approaches for determining gene function and for modeling human tumorigenesis. The first approach is to model human spontaneous cancers using a conditional chromosome engineering approach for generating specific chromosome deletions somatically. This strategy will allow us to design mouse models that more accurately reflect human spontaneous cancers in which a genomic alteration within a rare somatic cell leads to tumor formation. Another approach that we have been developing in a collaborative effort with David Largaespada's laboratory (University of Minnesota, Minneapolis) combines chromosome engineering with transposon-mediated mutagenesis. The strategy makes it possible to thoroughly examine a defined genomic interval to uncover novel cancer genes.

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

E. Garcia, X. Guo, W. Keyes, C. Papazoglu

Goal: To uncover molecular and genetic mechanisms whereby p63 *regulates development, cancer, and aging.*

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

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

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

We have also generated mouse models that mimic the human developmental disease syndrome Ectrodactyly, Ectodermal dysplasia, Clefting (EEC) syndrome. EEC is caused by specific mutations in the DNA-binding domain of *p63*; interestingly, these mutations correlate precisely with cancer-causing mutations in the *p53* gene. To model EEC, we used gene targeting to replace the normal copy of *p63* with a version of *p63* that has an EEC mutation. Mice carrying the EEC mutation have a phenotype that correlates with the clinical features found in EEC patients. Further characterization of this model will allow us to more thoroughly understand the underlying cause of the pathogenesis of EEC and will form a basis for better treatment of patients afflicted with this disease.

In a collaborative effort with Gerry Cunha's laboratory (University of California, San Francisco), we used the *p63* mouse models we have developed to determine that *p63* dictates epithelial cell fate (Fig. 1). When tissue recombination experiments between ectoderm and mesenchyme were performed, it was shown that *p63* expression drives the uncommitted Mullerian epithelial cell to commit to the cervicovaginal lineage, whereas in *p63*'s absence, these cells were destined to become uterine epithelia. The ability to drive epithelial cell fate highlights *p63*'s master role in differentiation.

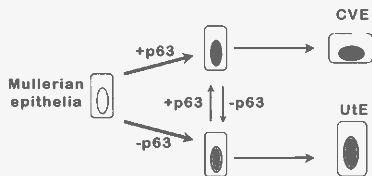

FIGURE 1 *p63* dictates epithelial cell fate. The female reproductive tract is lined with two distinct types of epithelia. The cervicovaginal epithelia that line the cervix and vagina perform a protective role, whereas the uterine epithelia that line the uterus perform a glandular/secretory role. Although these two types of epithelia are distinct, they arise from a common precursor: the Mullerian epithelial cell. In response to *p63* activation, these undifferentiated cells commit to the cervicovaginal lineage, whereas in *p63*'s absence, these cells are destined to differentiate into uterine epithelia. Tissue recombination experiments indicate that *p63* is a master controller of this differentiation program.

Our analysis of the different *p63* models that we have developed have recently uncovered an unanticipated link between cancer and aging. We found that unlike *p53*-deficient mice, mice with compromised *p63* levels were not cancer-prone. In fact, we found that reduced levels of *p63* functioned to decrease tumor incidence in the highly cancer-prone *p53*-compromised mouse. Indeed, the rare tumors that developed in *p63*-compromised mice were molecularly and histologically distinct from those of *p53*-deficient mice. Thus, *p63* does not have a role in tumor suppression equivalent to that of *p53*. In fact, decreased *p63* function appears to be tumor protective.

Although we found that *p63*-deficient mice were not tumor prone, we discovered that they aged prematurely. Mice with decreased *p63* levels had a 23% reduction in life span relative to wild-type mice and showed features of accelerated aging. When we analyzed the effect of ablating *p63* specifically within the skin of adult mice, we found that aging was dramatically accelerated: These mice developed baldness, skin defects, curvature of the spine, weight loss, etc. Interestingly, we found that *p63* loss correlated with a striking increase in the number of senescent cells, both in vitro and in vivo. Thus, *p63* deficiency appears to provide tumor protection at the cost of accelerating the aging process.

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TUMOR ANGIOGENESIS

V. Mittal D. Nolan S. Gupta
A. Mellick E. Heikamp
W. Xue

The presence of several genetic and epigenetic alterations in the genome of a cancer cell has compromised the effectiveness of anticancer therapeutics. In contrast, the genetically stable tumor-associated vasculature, intrinsic for the survival, proliferation, and metastasis of a majority of tumors, has been regarded as an attractive target for cancer therapy. However, clinical trials with antiangiogenic factors for the treatment of human cancer have not been as effective, suggesting that tumor angiogenesis might be orchestrated by a more complex set of growth factors and collaboration with as yet unrecognized cell types. Identifying and targeting these cells may be vital key to successful antiangiogenic therapy.

Indeed, emerging evidence suggests that bone-marrow-derived endothelial progenitor cells (EPCs) contribute to de novo blood vessel formation in the adult. EPCs are mobilized from the bone marrow into the peripheral circulation, recruited, and incorporated into sites of active neovascularization during tissue ischemia, vascular trauma, or tumor growth. Notably, preclinical and pioneering clinical studies have shown that introduction of these progenitor cells can restore tissue vascularization after ischemic events in limbs, retina, and myocardium. These initial findings have provoked extensive follow-up studies—some casting doubts on the biological significance and even the contribution of the progenitor cells to the tumor vasculature.

Little is known about the critical role of EPCs in the progression of tumors, and many questions remain to be addressed to understand the molecular and cellular mechanisms governing EPC mobilization, differentiation, and incorporation into functional tumor vasculature. For example, what constitutes a homogeneous EPC population in the total bone marrow? How are the EPCs mobilized into the peripheral circulation in response to tumor growth factors, and how do they home in to the sites of the tumor and incorporate into the functional vasculature? Will blocking the mobilization and incorporation of EPCs prevent tumor progression? In the last year, the focus of research in our laboratory has been to answer some of these challenging questions by applying powerful genomic technologies to mouse genetic models.

To understand how tumors direct the expansion, mobilization, and incorporation of EPCs into the functional tumor neovasculature, we have established a robust murine bone marrow transplantation (BMT) model. In this transplant model, green fluorescent protein (GFP)-marked donor bone-marrow-derived EPCs are transplanted into lethally irradiated recipients and tracked in the peripheral blood and in the tumor vasculature in vivo. Four weeks posttransplantation, fluorescence-activated cell sorting (FACS) analysis of blood showed greater then 85% GFP^+ cells, indicating efficient reconstitution of recipient hematopoiesis by the donor cells. We first investigated whether the transplanted EPCs can be mobilized from the bone marrow into the peripheral circulation in response to tumor challenge. Upon tumor challenge, a sevenfold increased mobilization of GFP^+ $VEGFR2^+$ cells that lacked the myelomonocytic marker CD11b /CD45 was observed as compared to unchallenged mice. We next evaluated whether these mobilized circulating endothelial precursor (CEP) cells were able to home in and incorporate into functional vessels in the tumor. Microscopic analysis of tumor sections for colocalization of GFP and multiple endothelial cell and pericyte markers (e.g., PECAM, VE-cadherin, NG2, and PDGF) showed significant incorporation of GFP^+ transplanted cells into the tumor vasculature. A more detailed quantitation suggested that approximately 60% of these cells are derived from the donor bone marrow. These cells were present in multiple architectural forms, such as in tubes, vessels, single cells, and strings of cells. Detailed characterization of these cells is in progress. Optical sectioning of serial sections confirmed the colocalization of GFP and CD31 signals within the same individual cell, suggesting that the incorporated endothelial cell was derived from the transplanted EPCs.

We were next interested in understanding if the contribution of the bone marrow precursors had any biological significance in angiogenesis-mediated tumor growth and metastasis. Toward this goal, we

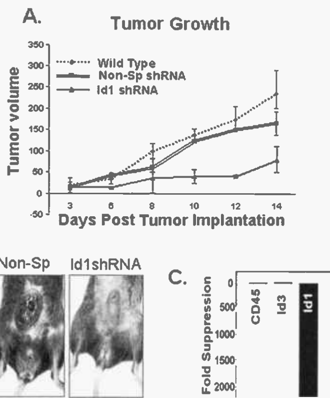

FIGURE 1 Blocking EPC-mediated tumor angiogenesis by RNAi in vivo. (*A*) Tumor growth in wild-type, nonspecific shRNA and *Id1*-specific shRNA transduced lineage negative bone marrow cells. (*B*) Tumors on day 14 in mouse transplanted with bone marrow precursors expressing a nonspecific shRNA (*left*), and an *Id1*-shRNA (*right*). (*C*) RT-PCR analysis showing fold suppression of *Id1*, *Id3*, and a myelomonocytic marker CD45 mRNA in bone marrow as a result of *Id1* shRNA. The comparison is with the bone marrow expressing a nonspecific shRNA.

identified that transcription factors *Id1* and *Id3* were up-regulated in tumor/VEGF-challenged bone marrow precursors. We asked if up-regulation of *Id* gene expression was critical for EPC-mediated tumor angiogenesis and would suppression of *Id* gene expression by RNA interference block EPC function. Effective short hairpin RNAs (shRNAs) targeting *Id1* and *Id3* mRNA were identified by using a rapid and quantitative screening approach developed previously in the laboratory (Kumar et al., *Genome Res. 13:* 2333 [2003]). shRNA-mediated suppression of *Id* gene expression resulted in a dramatic reduction in endothelial tube formation in matrigel assays, phenocopying the angiogenic defect observed in *Id* knockout mice.

Strikingly, animals with bone marrow transduced with lentivirus-mediated *Id1*-specific shRNA showed significant delay in tumor growth (40% reduction), as compared to animals transplanted with bone marrow expressing a nonspecific shRNA or wild-type animals that had not undergone bone marrow transplantation. The tumors were next characterized to determine the impact of shRNA-mediated *Id* gene suppression on the contribution of donor-derived cells in establishing the neovasculature. Analysis of tumor sections showed a dramatic reduction in GFP$^+$ endothelial cells in the presence of *Id1* shRNA, when compared to animals carrying wild-type GFP$^+$ bone marrow or nonspecific shRNA expressing bone marrow. RT-PCR (reverse transcriptase–polymerase chain reaction) analysis of bone marrow cells showed that the *Id1*-specific shRNA caused a dramatic reduction in the levels of *Id1* mRNA in vivo. Suppression of *Id1* did not affect normal hematopoiesis (levels of CD45 mRNA, a myelomonocytic marker remained unchanged), in agreement with published observations.

In summary, we now have evidence that not only supports and extends the concept for the incorporation of bone marrow precursor cells in the tumor neovessels, but also establishes their functional importance in angiogenesis-mediated tumor growth. We have identified genes that mediate recruitment of bone marrow precursors to the site of active angiogenesis. Strikingly, shRNAs targeting these genes in the bone marrow precursors resulted in a rapid delay in tumor growth as a result of reduced bone marrow contribution to tumor neovascularization.

We are currently identifying pure EPC populations resident in the total bone marrow, 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.

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CANCER GENES

S. Powers D. Mu

In October of this past year, we started our laboratory and began looking in human tumors for genomic alterations that are likely to be causally involved in cancer progression.

Besides our own initiatives, we are keyed up by the interactive and collaborative spirit of CSHL, and began what we expect will be productive collaborations with different groups here at CSHL. Our first collaborative effort was with Robert Lucito and Scott Lowe, who had previously teamed up to look at secondary genetic alterations that occur in mouse liver tumors initiated by overexpression of *MYC* in combination with suppression of *p53*. Rob and Scott had found a recurrent amplicon in mouse tumors that looked virtually identical to an amplicon we discovered in human liver tumors. We are hopeful that this result is an indication that other human genetic lesions we discover can be functionally examined in the right tissue type and in the appropriate environment, rather than relying solely on human cancer cell line models for functional analysis.

Our first initiative was to demonstrate the utility of ROMA (representational oligonucleotide microarray analysis) in breast cancer gene discovery. Using data we generated ourselves, together with data from Mike Wigler's laboratory here at CSHL, we tracked down a novel recurrent amplicon at chromosomal region 17q22. This amplicon is found in some breast tumors that do not harbor *ERBB2/HER2* amplification, but more often, it is found in tumors harboring *ERBB2/HER2* amplification. We are utilizing short hairpin RNA (shRNA) from Greg Hannon's library here at CSHL to examine the effects of shRNA-mediated knockdown of overexpressed genes from the amplicon in appropriate breast cancer cell lines, and collaborating with Senthil Muthaswamy (also at CSHL) to examine the effects of overexpressing this candidate oncogene along with *ERBB2* in the mouse model his group has developed for breast cancer.

We also initiated ROMA analysis of gene-dosage effects in colon cancer. Based on our initial look at 75 colon tumors and cell lines, the major application in colon cancer gene discovery will be identification of candidate tumor suppressors. We say this because unlike breast cancers, which often have highly localized amplicons that can pinpoint candidate amplified oncogenes, colon tumors often have many highly localized deletions, many of which appear to be homozygous. Curiously, we did not find localized deletions that affected either *APC* or *TP53*, the two most prevalently mutated cancer genes in colon cancer. Nor did we find any larger deletions affecting these genes, which implies that the allelic loss which accompanies point mutations in these two suppressor genes occurs by a mechanism that does not affect DNA copy number. However, we did find localized deletions that affected *DPC4* and *MSH2* (Fig. 1), two well-established tumor suppressor genes.

We are thus optimistic that we can identify novel colon cancer tumor suppressors. As a cautionary note, the cancer field is littered with candidate tumor suppressor genes that are not commonly accepted as being clinically or functionally relevant. A major challenge this coming year will be to focus our research so that we can identify truly relevant tumor suppressor genes.

One of the major hopes people have for the utility of ROMA is that it will lead to the development of DNA-based diagnostic tests that can provide early detection or help guide specific treatment choices. Currently, DNA-based diagnostic tests for early detection of colon cancer catch a little over 50% of what is

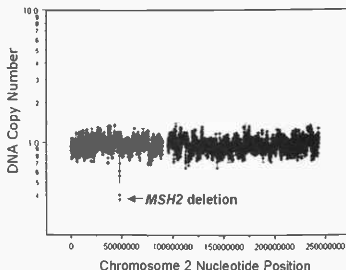

FIGURE 1 Chromosomal plot of DNA copy number along chromosome 2 for a colon tumor sample. Note the sharp deletion affecting only *MSH2*.

captured by the standard invasive test, which is not enough to change clinical practice. The current DNA-based test detects sequence variation in four well-established colon cancer genes (including *TP53*, *KRAS*, and *APC*) and could be greatly strengthened by including detection of the most commonly homozygously deleted regions discovered by ROMA.

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David Mu, research investigator, Scott Powers' lab.

MAMMALIAN CELL GENETICS

M. Wigler	J. Alexander	J. Healy	A. Reiner
	M. Chi	J. Hicks	M. Riggs
	D. Esposito	E. Leibu	L. Rodgers
	H. Grasmo-Wendler	L. Muthuswamy	J. Sebat
	V. Grubor	N. Navin	J. Troge

This year marks a major turning point in the development of our laboratory. Traditionally, we have been a cancer research laboratory. Even during the period when the major problems we studied were embodied in model organisms—the yeasts, *Saccharomyces cerevisiae* and *Schizosaccaromyces pombe*—our effort was to understand abnormal growth regulation as a consequence of altered signal transduction. Although cancer is still one of our major efforts, we have taken on a new problem, the genetics of complex human diseases. Representational oligonucleotide microarray analysis (ROMA), the tool we developed for exploring "copy-number differences" between normal and cancer genomes, can equally well be used to explore the genetic differences among humans that may predispose individuals to medical disorders.

ROMA reveals that there are large differences in the number of genes in individual human genomes. One distinction of the complex metazoans is the size of gene families. Species evolve, at least in part, by gene duplication followed by specialization. It should not surprise us that we find this process alive and well in humans (Sebat et al. 2004). What does surprise us, given the intense scrutiny of the human genome by other methods, is that its extent was not previously appreciated. We expect to find differences in the number of copies of genes that account for disease susceptibilities, and we are determining if these differences contribute to autism.

BREAST CANCER AND LEUKEMIAS

We have now examined the abnormalities in a modest series of primary breast cancers. The bulk of our study comes from our collaborator Anders Zetterberg at the Karolinska Institutet in Stockholm, Sweden, who has framed the following question: Can we correlate the prognosis of a cancer with its genomic profile? The simple answer appears to be yes. There are more genomic lesions in the patients that succumb to their cancers than in those that survive, as well as specific loci that when unperturbed appear to correlate with survival. We seek to extend and confirm these observations in a larger series of experiments.

The same set of experiments have confirmed imbalances at well-known loci that contain oncogenes and tumor suppressor genes, but they have also revealed common imbalances at loci where there are not known candidate tumor genes. We are in the process of preparing to determine by correlation analysis specific patterns of imbalances that might suggest the pathways that are disturbed in cancer development, and the order in which these disturbances arise. Preliminary results indicate that a few very common lesions characterize the early stages of breast cancers.

In a collaboration with Nick Chiorazzi at North Shore–L.I.J. Research Institute, we have taken similar approaches to study chronic lymphocytic leukemia (CLL). There are fewer lesions present in CLL than in advanced stage breast cancer, but again, as in early stage breast cancer, there are a few common lesions. We wish to extend these studies to a larger series, to gain a foothold in predicting the outcome of the disease.

LARGE-SCALE POLYMORPHISM IN HEALTHY AND IMPAIRED HUMANS

As reported last year, we have applied our methods to the comparison of genomes from apparently healthy humans and humans with various disorders. There are a large number of extensive regions of copy-number variation between any two humans, approximately equal amounts of deletions and duplications. We have now examined on the order of several hundred individuals and find that we detect on the order of a dozen differences of 100 kb and larger between any two people. These regions contain one gene on average. We estimate that we see about half the lesions of this size or larger, and lesions that are smaller largely escape our present methods. Although copy-number polymorphisms exist throughout the chromosomes, they are conspicuously rare on the X chromosome and on the gene-rich chromosome 19.

A statistically significant difference appears to exist between autistic individuals and normal control populations: There is an increase both in the total amount of genome lost in autistics and in the size of their largest deletions. This is a property of the population as a whole, not one of individuals per se. Nevertheless, we do see genetic differences in autistics at specific loci, and in particular at loci that have been previously linked to mental disorders such as Tourrette's Syndrome and obsessive-compulsive disorders.

Considerably more effort is required to validate the significance of the differences we observe. We will soon apply similar methods to schizophrenia, congenital heart disease, and a variety of other human impairments.

TECHNOLOGY DEVELOPMENT

As before, we strive to improve both the resolution of our methods and their range of application. These efforts are largely focused on two objectives: increasing the density of probes that can be arrayed, and thus the total number of loci that can be observed, and diminishing the number of cells required for analysis. The former goal will enable us to screen smaller lesions that might upset gene function, and we are pursuing technology development in combination with NimbleGen Systems, the company that fabricates our arrays. A number of computational-algorithmic problems have needed to be solved, as well as the development of new laboratory protocols. In terms of "miniaturization," we have preliminary results indicating clear success with as few as 100 cells, and analysis of single cells is not beyond our reach. Such methods would enable us to examine the role of somatic rearrangement in aging, development, and in the evolution of the malignant phenotype.

All of our methods must transpire in a milieu of computationally based informatics processing, and so an increasingly large percentage of our effort is expended on creating mathematical tools for data analysis, and creating environments in which the large number of experiments and results of analysis can be viewed. Foremost in these efforts has been the development of reliable methods of "segmentation" (Daruwala et al. 2004; Olshen et al. 2004; Sebat et al. 2004). In these matters, we have been aided by collaborators (see authors of publications). The results of segmentation are validated by fluorescent in situ hybridization in collaboration with Dr. Anders Zetterberg's group at the Karolinska Institutet.

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CANCER: CELL BIOLOGY

The most basic requirement of living organisms is their ability to copy themselves by duplicating their genetic material and then dividing it up into two new daughter cells through cell division. Each human cell contains about six feet of DNA that must be precisely coiled and tightly condensed into chromosomes before cell division can take place. Tatsuya Hirano studies how cells organize and manage chromosome structure in preparation for cell division. His current studies are focusing on two protein complexes, called condensin I and II, that have key roles in compacting DNA and ensuring that each daughter cell receives one, and only one, copy of each chromosome. Hirano recently collaborated with CSH Fellow Terence Strick to precisely measure the forces that act on DNA as it is compacted by condensin I. They discoverd that condensin I actively compacts DNA through an energy-dependent process that reversibly introduces loops along the DNA.

Bruce Stillman is focusing in part on how cells are able to efficiently copy or "replicate" their chromosomal DNA in preparation for cell division. To copy their DNA, cells rely on large complexes of proteins that recognize specific regions within the genome as start sites or "origins" of DNA replication. Stillman studies a protein complex called the origin recognition complex (ORC) that attaches specifically to those sites and then triggers DNA replication. Recent work in his lab has established that particular ORC proteins have additional roles in coordinating chromosome duplication with cell division. In cancer cells, the mechanisms that regulate cell division are compromised. Therefore, Stillman's findings concerning how normal cells coordinate chromosome duplication and cell division are revealing clues to how these processes go awry in cancer.

Viruses are masters of cellular manipulation. By studying how the human papillomavirus co-opts the DNA replication machinery of the cells it infects into making more virus, Arne Stenlund is uncovering mechanisms that govern human DNA replication. He has revealed how the viral initiator protein E1 recognizes and engages the origin of DNA replication and has demonstrated that the E1 protein binds tightly to the origin through two different DNA-binding activities present in the same protein.

David Spector and his colleagues have developed the first system for viewing how "the Central Dogma" of biology unfolds in its entirety—from DNA to RNA to protein—within living cells. The researchers created a fluorescence microscopy imaging system in which the DNA near an inducible gene is labeled green, the messenger RNA encoded by the gene is labeled yellow, and the protein encoded by the messenger RNA is labeled blue. The system is then used to capture time-lapse images as the inducible gene is switched on: First, the DNA architecture in the region of the gene becomes less compacted. Next, RNA appears, is spliced in the nucleus, and subsequently exported to the cytoplasm. Finally, the protein appears. Spector's group has used the system to detect specific events that transform the architecture of chromosomes from a transcriptionally silent "off" state to an actively transcribed "on" state. This work has revealed fundamental information about how genes are switched on and off in normal cells and is being used to investigate how the production of RNA and proteins is altered in cancer or other diseases.

Understanding how cancer cells grow and spread requires studying their interactions with the cells that surround them in three-dimensional space. To this end, Senthil Muthuswamy uses three-dimensional cell cultures (in contrast to traditional two-dimensional cultures) that mimic the normal glandular structures of human breast tissue. He then activates cancer-promoting genes and studies how this activation disrupts normal breast tissue architecture and cell growth controls. When Muthuswamy's group activated the *ErbB2* oncogene, they noticed that cells lost the normal junctions between neighboring cells, began to proliferate, and generated a disorganized mass of cells. Muthuswamy is exploring the role of these changes in cell architecture in the early stages of invasive breast cancer.

Like Muthuswamy, David Helfman (now at the University of Miami) explores how cancer cells manage to break free from their biological moorings and metastasize to other parts of the body. In collaboration with Andrei Bakin (Roswell Park Cancer Institute), Helfman's group recently showed that hyperactivating the *Ras* oncogene pathway disrupts the formation of cytoskeletal elements that normally prevent cells

from spreading to other parts of the body. These findings could ultimately lead to the development of new strategies for preventing the spread of cancer cells.

Many times, proteins that are first described as having a role in a particular process turn out to be important for other, seemingly unrelated processes. Linda Van Aelst has previously revealed how proteins—called Rho and Rac GTPases—function in cell proliferation, cell/cell adhesion, and tumor invasiveness. Recently, Van Aelst and her colleagues discovered a role for regulators of Rho and Rac GTPases in brain development. They found that oligophrenin-1, an activator of Rho GTPase known to be involved in X-linked mental retardation, is required for the proper shape and functioning of brain neurons. In related work, Van Aelst has established that DOCK7, a newly discovered activator of Rac GTPases, is required during early stages of brain development for the projections from neurons called axons to form properly.

Nicholas Tonks and his colleagues were the first to show that abnormalities in proteins called protein tyrosine phosphatases (PTPs) can lead to cancer. Tonks has uncovered roles for PTPs in virtually every aspect of the life of a cell from proliferation, to cell motility, to cell architecture. Recently, Tonks has been studying a process whereby cell signaling results in the production of reactive oxygen species (ROS). The production of reactive oxygen species typically leads to the inactivation of PTPs, thereby removing the "brakes" that PTPs normally provide and allowing particular signaling pathways to proceed. Tonks is investigating these mechanisms and their therapeutic implications for cancer and other diseases.

Santhosh Vadivelu, postdoc, Bruce Stillman's lab.

THE CYTOSKELETON IN CANCER

D.M. Helfman L. Connell

After almost 23 years at Cold Spring Harbor Laboratory, I took a faculty position in July 2004, at the Sylvester Comprehensive Cancer Center and Department of Cell Biology and Anatomy in the Miller School of Medicine at the University of Miami. I first arrived at Cold Spring Harbor in the fall of 1981 as a postdoctoral fellow, and for more than two decades, it has been an exciting and rewarding environment in which to carry out research. It was a privilege to be part of such a unique place, and I wish only the best in the future for the Laboratory and its members. Although I cannot mention all of the people whom I had the pleasure to interact with, I would like to take this opportunity to thank my postdoctoral advisors Jim Feramisco and Steve Hughes for giving me the opportunity to work in their labs at a time when molecular biology was not done using kits, Jim Watson for his support during my early years as a staff member, and Barbara McClintock and Yasha Gluzman for their friendship and words of wisdom.

My lab is focused on understanding various aspects of oncogene-mediated changes in actin filament dynamics of tumor cells. Alterations in the actin-based cytoskeleton are an integral part of the neoplastic phenotype. We are investigating the signaling pathways by which specific components of actin structures are targeted and deregulated by oncogenes and, more importantly, how the accompanying changes in actin filament organization contribute mechanistically to oncogenesis. Studies from our lab have demonstrated that alterations in the actin cytoskeleton result from activation and inactivation of specific signaling pathways that are part of the oncogenic program. These studies are providing valuable new insights into the regulation of the actin cytoskeleton by oncogenes and how changes in the actin cytoskeleton contribute to enhanced cell motility and invasiveness, as well as aberrant signaling of cancer cells. Furthermore, specific components of the cytoskeleton might serve as a target for the development of prognostic, diagnostic, and therapeutic targets in cancer. Below is a brief description of our studies.

Role of TGF-β Signaling in Oncogenesis and Tumor Suppression via Regulation of Actin Filament Dynamics in Breast and Cervical Cancers

D.M. Helfman

The transforming growth factor-β (TGF-β) signaling pathway is a major cellular growth inhibitory and proapoptotic pathway in normal epithelial, endothelial, hematopoietic, and other cell types. However, clinical and experimental studies indicate that metastatic cancers of the breast and other tissues express elevated levels of TGF-β that appear to support the metastatic behavior of tumor cells. This apparent paradox has been associated with a progressive decline in the antitumorigenic function and a gain of pro-tumorigenic activities of TGF-β, including induction of epithelial to mesenchymal transition (EMT), tumor cell migration and invasion. The actin cytoskeleton has a central role in the regulation of cellular processes linked to metastasis, including cell proliferation, apoptosis, anchorage-independent cell growth, cell migration, and invasion. Prolonged exposure of normal epithelial cells with TGF-β results in the formation of stress fibers and focal adhesions, accompanied by inhibition of cell motility and induction of apoptosis. Thus, the loss of TGF-β-induced stress fibers is an essential characteristic of a prometastatic conversion of TGF-β function. Establishing the cellular targets involved in TGF-β signaling is critical to understanding cancer progression. During the past year, in collaboration with Dr. Andrei Bakin (Roswell Park Cancer Institute, Buffalo, New York), we discovered a critical role for a family of actin-filament-binding proteins—tropomyosins (TMs)—required for TGF-ß regulation of the actin cytoskeleton in breast and cervical cancers. We demonstrated that expression of TMs mediated by Smad and p38Mapk signaling is required for TGF-β regulation of stress fibers and inhibition of cell motility. We showed that

the oncogenic Ras-ERK pathway antagonizes TGF-β induction of stress fibers by suppressing expression of TMs. These results suggest that loss of TGF-β-induced stress fibers is an essential characteristic of a pro-metastatic conversion of TGF-β function and that the aberrant regulation of TMs is a critical feature in breast cancer. We hypothesize that the loss of TGF-β induction of stress fibers is an essential characteristic of a pro-metastatic conversion of TGF-β function and that restoration of this response represents a potential target for the development of effective antimetastatic therapies.

Distinct Roles of MLCK and ROCK in the Regulation of Epithelial Cell Survival

L. Connell

Recent studies have demonstrated the importance of intact actin filaments for cell survival. Myosin II activation is essential for stress fiber and focal adhesion formation and is implicated in integrin-mediated signaling events. To determine the role of myosin II in cell survival, we studied whether inhibition of myosin light-chain kinase (MLCK) or Rho-kinase (ROCK) leads to apoptosis in both normal and *ras*-transformed MDCK and MCF-10A epithelial cells. Treatment of cells with pharmacological inhibitors of MLCK (ML-7 and ML-9), or expression of dominant-negative MLCK, led to apoptosis in normal and transformed cells. In contrast, treatment of cells with the ROCK inhibitor (Y-27632) did not induce apoptosis in these cells. Apoptosis following inhibition of myosin II activation through MLCK is likely meditated through the death receptor pathway because expression of CrmA or a dominant-negative FADD (Fas-associated death domain) blocked apoptosis. In addition, apoptosis observed after MLCK inhibition is inhibited by pretreatment of cells with integrin-activating antibodies, suggesting the participation of an integrin-dependent signaling pathway. These studies demonstrate a novel role for MLCK in the generation of antiapoptotic signals in both untransformed and transformed epithelial cells and support previous work suggesting distinct cellular roles for ROCK- and MLCK-dependent regulation of actomyosin contractility.

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HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano N. Aono A. Losada
R. Gandhi I. Onn
P. Gillespie T. Ono
M. Hirano

The long-term goal of our research program is to understand the molecular mechanisms of mitotic chromosome segregation in eukaryotic cells. Our current efforts are focused on studying two multiprotein complexes, cohesin and condensin, that have central roles in sister chromatid cohesion and condensation, respectively. At the heart of the two complexes lie members of a large family of chromosomal ATPases, the structural maintenance of chromosomes (SMC) family. SMC proteins are also conserved in bacterial and archaeal species, implicating the existence of an evolutionarily conserved theme of higher-order chromosome organization and dynamics. To gain insight into how SMC proteins work both in vitro and in vivo, we take multidisciplinary approaches including biochemistry, cell biology, genetics, and biophysics.

Spatial and Temporal Regulation of Condensins I and II

T. Ono, T. Hirano [n collaboration with Y. Fang and D. Spector, Cold Spring Harbor Laboratory]

Our previous studies showed that vertebrate cells have two different condensin complexes (condensins I and II) that make distinct contributions to metaphase chromosome architecture. During the past year, we have shown that the spatial and temporal distribution of condensins I and II are differentially regulated during the cell cycle in HeLa cells. Condensin II is predominantly nuclear during interphase and contributes to early stages of chromosome assembly in prophase. In contrast, condensin I is sequestered in the cytoplasm from interphase through prophase and gains access to chromosomes only after the nuclear envelope breaks down in prometaphase. The two complexes alternate along the axis of metaphase chromatids, but they are arranged into a unique geometry at the centromere/kinetochore region, with condensin II enriched near the inner kinetochore plate. This region-specific distribution of condensins I and II is severely disrupted upon depletion of aurora B, although their association with the chromosome arm is not. Depletion of condensin subunits causes defects in kinetochore structure and function, leading to aberrant chromosome alignment and segregation. Our results suggest that the two condensin complexes act sequentially to initiate the assembly of mitotic chromosomes and that their specialized distribution at the centromere/kinetochore region may have a crucial role in placing sister kinetochores into the back-to-back orientation.

Real-time Detection of Single-DNA-Molecule Compaction by Condensin I

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

To understand how condensin I works at a mechanistic level, we have performed single-molecule DNA nanomanipulation experiments. In these experiments, a linear DNA molecule is tethered at one end to a glass surface and at the other end to a small magnetic bead. Permanent magnets are used to pull on and rotate the bead, allowing us to quantitatively control the mechanical constraints applied to the DNA. The three-dimensional position of the magnetic bead is determined using video microscopy and real-time particle tracking algorithms, allowing us to determine the end-to-end extension of the DNA. By using this sophisticated device, we demonstrated for the first time that condensin I, purified from *Xenopus* egg extracts, physically compacts DNA in an ATP-hydrolysis-dependent manner. A mitotically activated form of condensin, but not its interphase form, supports this compaction reaction. Discrete and reversible DNA compaction events are observed in the presence of competitor DNA when the DNA is subjected to weak stretching forces ($F = 0.4$ pN). The distribution of step sizes is broad and displays a peak at approximately 60 nm (~180 bp) as well as a long tail. Increasing the force to $F = 10$ pN drives

the system toward step-wise reversal of compaction. The distribution of step sizes observed upon disruption of condensin-DNA interactions displays a sharp peak at about 30 nm (~90 bp) as well as a long tail stretching out to hundreds of nanometers. Our results suggest that the condensin complex may induce DNA compaction by dynamically and reversibly introducing and stabilizing loops along the DNA.

Biochemical and Structural Dissection of Condensins I and II

I. Onn, M. Hirano, T. Hirano

Previous studies from our laboratory demonstrated that condensin I, purified from either *Xenopus* egg extracts or human tissue culture cells, is able to introduce positive superhelical tension into DNA in an ATP-dependent manner. However, functional contribution of each subunit to this activity remains to be determined. Moreover, it is unknown whether the newly discovered condensin II may have an activity similar to that of condensin I. To address these questions, we are taking two complementary approaches. First, we purify condensins I and II separately from HeLa cells and compare and contrast biochemical activities associated with these native complexes. Effects of Cdk1-dependent phosphorylation on these activities are also investigated. Second, we reconstitute condensins I and II from their recombinant subunits by using the baculovirus expression system. We have successfully constructed a panel of recombinant viruses that express individual subunits of the human complexes. Pairwise coexpression experiments, followed by specific immunoprecipitation, are in progress to make a comprehensive map of subunit-subunit interactions. Protocols are also being established for expressing and purifying holo- and subcomplexes of condensins I and II.

Mitotic and Nonmitotic Functions of Condensins in *Xenopus* Egg Extracts

N. Aono, T. Hirano

Accumulating lines of evidence from a number of model organisms suggest that the fundamental roles of condensins in chromosome organization and dynamics are far beyond the condensation of chromosomes during mitosis. We have started to explore nonmitotic functions of condensins by using *Xenopus* egg cell-free extracts. An initial effort is focused on asking whether condensins may be involved in the DNA replication and damage checkpoint pathways, as has been implicated from a genetic study in fission yeast. We are now testing how checkpoint kinases behave in response to double-strand DNA breaks, in the extracts that have been mock depleted or depleted of condensins. We are also interested in understanding how the two different condensin complexes, condensins I and II, are differentially regulated throughout the cell cycle. Potential roles of Cdk and non-Cdk mitotic kinases are being tested in the cell-free extracts.

Functional Contribution of Pds5 Proteins to Cohesin-mediated Cohesion

A. Losada, T. Hirano

The cohesin complex has a central role in sister chromatid cohesion during mitosis and meiosis. Previous genetic studies in fungi identified Pds5/BimD/Spo76 as an additional factor implicated in cohesion. During the past year, we have performed the biochemical and functional characterization of two Pds5-like proteins, Pds5A and Pds5B, from vertebrate cells. In HeLa cells, Pds5 proteins physically interact with cohesin and associate with chromatin in a cohesin-dependent manner. Depletion of the cohesin subunit Scc1 by RNA interference (RNAi) leads to the assembly of chromosomes with severe cohesion defects. A similar yet milder set of defects is observed in cells with reduced levels of Pds5A or Pds5B. In *Xenopus* egg extracts, mitotic chromosomes assembled in the absence of Pds5A and Pds5B display no discernible defects in arm cohesion, but centromeric cohesion is apparently loosened. Unexpectedly, these chromosomes retain an unusually high level of cohesin. Thus, Pds5 proteins seem to affect the stable maintenance of cohesin-mediated cohesion and its efficient dissolution during mitosis. We propose that Pds5 proteins have both positive and negative roles in sister chromatid cohesion, possibly by directly modulating the dynamic interaction of cohesin with chromatin.

Scc2 Couples Replication Licensing to Sister Chromatid Cohesion

P. Gillespie, T. Hirano

Previous genetic studies in yeast showed that Scc2, a HEAT-repeat containing protein, is required for the loading of cohesin onto chromatin. We have identified two isoforms of Scc2 in humans and *Xenopus* (termed Scc2A and Scc2B), which are encoded by a single gene but have different carboxyl termini created by alternative splicing. Both Scc2A and Scc2B bind to chromatin concomitant with cohesin during DNA replication in *Xenopus* egg extracts. Simultaneous immunodepletion of Scc2A and Scc2B from the extracts impairs the association of cohesin with chromatin, leading to severe defects in sister chromatid pairing in the subsequent mitosis. The loading of Scc2 onto chromatin is inhibited in extracts treated with geminin, but not with $p21^{CIP1}$, suggesting that this step depends on replication licensing but not on the initiation of DNA replication. Upon mitotic entry, Scc2 is removed from chromatin through a mechanism that requires cdc2 but not aurora B or polo-like kinase. Our results suggest that vertebrate Scc2 couples replication licensing to sister chromatid cohesion by facilitating the loading of cohesin onto chromatin.

Reconstitution of a Recombinant Cohesin Complex

P. Gillespie, T. Hirano

Despite recent progress in our understanding of the diverse cellular functions of cohesin, it remains elusive how this complex might work at a mechanistic level. To study the action of cohesin in great detail, we have reconstituted a four-subunit cohesin complex from its recombinant subunits by using the baculovirus protein expression system. We have also introduced a set of mutations into the SMC core subunits that would block the SMC ATPase cycle at three different stages. Ongoing functional assays using the wild-type and mutant complexes should address how the mechanical cycle of cohesin is coupled to its catalytic cycle. Critical comparison between the actions of cohesin and condensin will also be of great importance to obtain a comprehensive molecular picture of chromosome mechanics in eukaryotes.

Regulation of Centromeric Cohesion

R. Gandhi, T. Hirano

In vertebrate cells, most (if not all) of cohesin dissociates from chromosome arms during mitotic prophase, whereas a subpopulation of cohesin remains at centromeres until the metaphase-anaphase transition. We are interested in understanding how centromeric cohesion and arm cohesion are differentially regulated during the cell cycle. It is most likely that a large number of factors are involved in this regulation. Such factors may include direct modulators of cohesin (Scc2 and Pds5), inner centromeric proteins (e.g., Sgo/Mei-S332 and aurora B) and heterochromatin components (e.g., HP1). By combining cell biological and pseudogenetic (RNAi) approaches, we are trying to dissect this regulatory network in human tissue culture cells. This project will contribute to our understanding of the molecular mechanism that ensures the high-fidelity transmission of chromosomes during mitosis.

Mechanistic Analysis of a Bacterial SMC Protein Complex

M. Hirano, T. Hirano

SMC proteins are conserved not only in eukaryotes, but also in most (if not all) bacterial and archaeal species. We have been using the *Bacillus subtilis* SMC protein as a model system for studying the basic mechanism of action of SMC proteins. An emerging theme is that the action of SMC proteins is highly dynamic and plastic, possibly involving a diverse array of intramolecular and intermolecular protein–protein interactions. During the past year, we have constructed and characterized a large number of mutant proteins that allow us to dissect the complex behaviors of SMC proteins in vitro. In particular, we have successfully identified key amino acid residues that are essential for SMC's DNA binding. The information from this project will be useful for studying the actions of condensin and cohesin, because most of the key residues identified in the bacterial SMC protein are invariable among eukaryotic SMC proteins.

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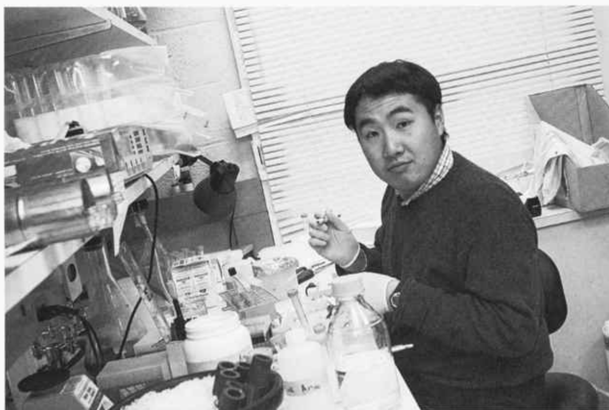

Nobuki Aono, postdoc, Tatsuya Hirano's lab.

EPITHELIAL CELL BIOLOGY AND CANCER

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EARLY EVENTS IN BREAST CANCER

Disruption of epithelial cell architecture and loss of proliferation control are thought to be early events in cancer. Changes in tissue architecture and proliferation rates are the primary tools used by pathologists to categorize early lesions. Although we understand a great deal about the mechanisms that control proliferation, our understanding of the mechanisms that control epithelial cell architecture is limited.

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

Normal breast epithelial cells, present in glandular structures in vivo, have low proliferation rates, whereas epithelial cells present in hyperplastic lesions or carcinoma have higher proliferation rates, suggesting that proliferation control is lost early in carcinoma. Mammary epithelial cells (MECs) grown in three-dimensional (3D) matrices form structures that share properties with resting acini in vivo such as low proliferation rates and empty central lumen space. In contrast, breast-tumor-derived cells neither form acini-like structures nor undergo proliferation arrest when placed under 3D culture conditions, suggesting that the mechanisms that regulate 3D morphogenesis are lost during transformation.

The ErbB family of oncogenic receptor tyrosine kinases contains four members; namely, ErbB1 (epidermal growth factor receptor/human epidermal growth factor receptor [HER]), ErbB2 (HER-2/Neu), ErbB3, and ErbB4. ErbB2 overexpression and/or amplification has been implicated in a number of epithelial cancers, including those of the breast, ovary, and prostate. Amplification of ErbB2 is observed in 20–30% of breast cancers and is correlated with poor clinical prognosis in node-positive patients.

Herceptin, an antibody targeted against ErbB2, is used as a therapeutic agent in adjuvant settings for patients who have breast cancers that express high levels of ErbB2. Despite the compelling evidence for the role of the ErbB family in breast cancer, it has been challenging to decipher the signaling and biological specificities of ErbB receptor activation because ligand binding induces both homo- and heterodimerization among the ErbB receptors, thus resulting in combinatorial interactions and complicating our ability to determine how different ErbB dimers transform mammary epithelia. To investigate how specific ErbB dimers regulate transformation of epithelial cells, we have developed a method to control dimerization and activation of ErbB receptors without contribution from endogenous receptors.

In addition, we have adapted cell culture approaches either to generate 3D acini-like epithelial structures or to generate suspended layers of epithelia. Both culture methods are characterized by the presence of a single layer of polarized, proliferation-arrested epithelial cells akin to those observed in resting ducts in vivo. Using a combination of controlled dimerization and modified cell culture systems, we investigate how activation of oncogenes, such as ErbB2, deregulates proliferation control and epithelial architecture.

How Is Epithelial Cell Polarity Lost during Transformation?

T. Haire, V. Aranda

In normal polarized epithelia, the boundary between the apical and basolateral membranes is defined by the presence of at least three classes of transmembrane proteins, namely, Occludins, Claudins, and Junctional adhesion molecules (JAM), collectively referred to as

the apical junction complex (AJC). The mechanism by which AJCs are positioned during polarization of epithelial cells involves a molecular complex containing the mammalian homolog of *Caenorhabditis elegans*, partitioning-defective 3 (mPar3)/mPar6/atypical protein kinase C/CDC42 or Rac (referred to as the Par complex). Consistent with this view, overexpression of mPar6, an inactive form of aPKC, active versions of CDC42 or Rac, cause either a delay or disrupt formation of AJC. Although the Par protein complex is critical for establishment of cell polarity, it is not known whether it is affected by oncogenic changes that disrupt polarity during cancer progression.

We have defined the steps for oncogene-induced disruption of polarity and, in doing so, discovered that mislocalization of an AJC-associated protein, ZO-1, begins at junctions that involve three cells (tricellular junctions) and not bicellular junctions. We have also shown that almost 60% of primary human breast cancers overexpressing ErbB2 have aberrant localization of ZO-1 at tricellular corners, suggesting that we have uncovered a novel target for oncogene action both in vitro and in vivo. We are now investigating the mechanisms by which oncogenes such as ErbB2 disrupt epithelial cell polarity.

Regulators of Epithelial Cell Polarity and Oncogenesis

M. Moore, A. Rosenberg

Studies in *Drosophila melanogaster, C. elegans*, and mammals have identified three protein complexes that are important for establishment of epithelial cell polarity, namely, the Par protein complex (Par3/Par6/aPKC/CDC42), which regulates formation of apical junctions; the Crumbs complex (Crumbs/Pals/PATJ), which regulates apical polarity; and the Scribble complex (Scribble, Discs large [Dlg] and Lethal [2] giant larvae [Lgl]), which regulates basolateral polarity.

Loss-of-function mutation analyses in *Drosophila* suggest that epithelial architecture can induce uncontrolled proliferation of epithelial cells. For instance, mutation of genes that control epithelial cell polarity such as *lgl, dlg*, and *scrib* not only result in loss of epithelial cell polarity, but also induce dramatic overgrowth of epithelial cells of imaginal disc and follicular epithelium. In addition, a loss-of-function mutation in the *Drosphila Fat* gene (fly homolog of human E-cadherin) results in neoplastic growth. These observations argue that proper epithelial architecture serves as a proliferation control signal in vivo and that loss of such a signal results in uncontrolled proliferation.

We are analyzing the role regulators of polarity have in human breast cancer by investigating whether polarity genes are amplified or deleted in breast cancers and whether they effect transformation of mammary epithelial cells in cell culture and mouse models.

How Does ErbB2 Induce Disruption of Epithelial Cell Polarity?

A. Lucs

Activation of ErbB2 induces disruption of apical-basal polarity of epithelial monolayers. Dimerization of ErbB2 results in phosphorylation of five tyrosine residues in its cytoplasmic tail. These phosphotyrosine residues are the primary mediators of ErbB2's transforming ability and have been previously shown to differentially regulate transformation of cells in culture and induce tumors in mice. To understand how ErbB2 disrupts epithelial cell polarity, the five autophosphorylation tyrosine residues were mutated to phenylalanine residues and are being analyzed for their ability to disrupt polarity.

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

B. Xiang

Amplification/overexpression of ErbB2 occurs in almost 50% of ductal carcinoma in situ (DCIS) of the breast. We identified MCF-10A as a human breast epithelial cell line that harbors genomic changes observed in early lesions and hence serve as a good model for ErbB2 overexpression. Using MCF-10A cells overexpressing ErbB2, we investigated the mechanism by which activation of ErbB2 induces cell cycle progression. We demonstrated that MCF-10A cells undergo a G_1-phase cell cycle arrest during 3D morphogenesis and activation of ErbB2-induced proliferation by increasing cyclin E/Cdk2 activity, but not cyclin D1/Cdk4 activity. ErbB2 increased Cdk2 activity by coordinately up-regulating cyclin E and down-regulating the cell cycle inhibitor $p27^{kip1}$. RNA-interference-mediated knockdown of cyclin E and Cdk2, but not cyclin D1 and Cdk4, inhibited the ability of ErbB2 to disrupt 3D acini structures. Activation of

ErbB2 also regulated cyclin E expression in two genetically diverse cell lines (S1 and SkBr3) grown under 3D culture conditions. In addition, overexpression of ErbB2 correlated with high levels of cyclin E, and not cyclin D1, in a panel of breast-cancer-derived cell lines. Thus, using a breast epithelial cell culture model for DCIS, we demonstrate that cyclin E is a target for ErbB2-induced proliferation in premalignant breast cancer.

Do ErbB Homodimers and Heterodimers Differentially Regulate Epithelial Cell Proliferation and Polarity?

L. Zhang, B. Xiang

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

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

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

B. Xiang, L. Zhang, A. Rosenberg, I. Sujka

Mouse models serve as valuable tools to study tumor initiation, progression, and treatment. Transgenic mouse models of breast cancer have provided significant insight into our understanding of oncogenic potential, tumor latency, and the relationship between genes and tumor histology. Conventional transgenic and knockout mice do not lend themselves to large-scale analyses of gene interactions due the time and money required for generating multiple genetic combinations. In the era of RNA interference (RNAi), we are in a position to down-regulate expression of genes without having to generate mouse strains by the long process of homologous recombination. In addition, RNAi allows us to down-regulate large numbers of genes, and more importantly, it empowers us with the ability to generate hypomorphic alleles akin to the gene products with reduced activity observed in human tumors. Although generating transgenic mammary glands, in an otherwise wild-type animal, by transplanting cells engineered in culture into developing mammary glands has been attempted in the past, the use of this technology to study gene interactions has not gained general acceptance in part due to the technical challenges associated with delivering transgenes into primary epithelial cells in culture.

We have optimized the culture conditions to efficiently transduce retrovirus-expressing transgenes and in addition, use short hairpin RNA (shRNA)-expressing vectors to down-regulate expression of genes. Transplantation of engineered cells resulted in mammary structures that express the transgene (GFP) and, more importantly, combined overexpression of oncogenic Ras and down-regulation of the tumor suppressor p53 resulted in formation of tumors within 12 weeks, demonstrating that we can rapidly generate transgenic mammary glands and analyze gene interactions during mammary tumorigenesis.

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CELL BIOLOGY OF THE NUCLEUS

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L. Denis

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) characterizing the establishment of the gene expression machinery in daughter nuclei after mitosis and (2) examining a new paradigm for the regulation of gene expression through a nuclear retained RNA.

ESTABLISHMENT OF THE GENE EXPRESSION MACHINERY IN DAUGHTER NUCLEI AFTER MITOSIS

Upon completion of mitosis, daughter nuclei assemble all of the nuclear organelles necessary for the implementation of nuclear functions. We have been interested in how the nuclear speckle pattern (interchromatin granule clusters, IGCs) is established in daughter nuclei because these nuclear domains contain a significant fraction of the machinery essential for gene expression (Saitoh et al. 2004).

During the past year, we found that upon entry into daughter nuclei, members of two different classes of pre-mRNA splicing factors, small nuclear ribonucleoprotein particles (snRNPs) and serine-arginine (SR) proteins, do not immediately colocalize in nuclear speckles (Bubulya et al. 2004). SR proteins accumulated in patches around active nucleolar organizing regions that we refer to as NOR-associated patches (NAPs), whereas snRNPs were enriched at other nuclear regions. NAPs formed transiently, persisting for 15–20 minutes (Fig. 1) before dissipating as nuclear speckles began to form in G_1. In the absence of RNA polymerase II transcription, NAPs increased in size and persisted for at least 2 hours, with delayed localization of SR proteins to nuclear speckles. In addition, SR proteins in NAPs are hypophosphorylated, and the SR protein kinase Clk/STY colocalizes with SR proteins in NAPs, suggesting that phosphorylation releases SR proteins from NAPs and their initial targets are transcription sites.

In mammalian cells, it is not clear how pre-mRNA processing factors are activated to a splicing-competent state in newly forming nuclei that have not yet assembled nuclear speckles. Our current observations suggest that regions surrounding NORs are important sites for initially segregating SR proteins away from other splicing factors, to modify them or to allow them to interact before association with pre-mRNA transcripts or nuclear speckles. This raises questions for future study regarding how SR proteins and snRNPs follow two separate pathways to eventually become targeted to the same speckle regions within daughter

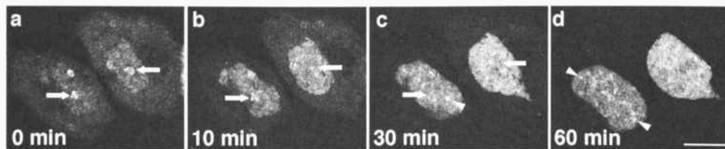

FIGURE 1 YFP-SF2/ASF localization was followed by confocal microscopy during telophase in living HeLa cells (*a–d*), where it transiently accumulates in bright NAPs (*a–c*, arrows) that persist for approximately 15–20 min, then disappear (*c*) as YFP SF2/ASF localizes to nuclear speckles (*c–d*, arrowheads). (From Bubulya et al. 2004.) Bar, 5 μm.

nuclei. It will be important to determine if SR proteins and snRNPs first meet at transcription sites before recycling of complexes through nuclear speckles. It also raises questions about how different nuclear compartments assemble/disassemble and communicate with one another as nuclear domains are established after mitosis.

The results of this study are key to building a time line of nuclear compartment assembly following mitosis. They also bring to light the fact that, in general, constituents of nuclear bodies, including pre-rRNA processing factors, components of Cajal bodies and PML bodies, and pre-mRNA processing factors, do not immediately localize to and/or assemble their resident bodies in daughter nuclei. Instead, there is a lag phase following mitosis as nuclear proteins first enter daughter nuclei and accumulate temporarily with proteins of similar function(s) before nuclear bodies (i.e., nuclear speckles and Cajal bodies) are established in G_1. These transient associations of subsets of proteins may be necessary for modification/maturation, or partial assembly of multimolecular subcomplexes before localization to nuclear bodies in G_1. The subsequent organization of nuclear compartments such as nuclear speckles would then provide further modification/ maturation and recycling/maintenance of protein complexes during interphase.

A NUCLEAR RETAINED RNA REGULATES THE LEVEL OF ITS PROTEIN-CODING PARTNER

In addition to harboring the cells' genome, the nucleus contains distinct substructures in the form of specialized domains/compartments that are characterized by the absence of delineating membranes, but contain defining sets of proteins (for review, see Spector, *Annu. Rev. Cell Biol. 9:* 265 [1993] and *J. Cell Sci. 114:* 2891 [2001]; Prasanth and Spector, in press). A dynamic interplay between these domains and/or their constituents and the genome is thought to foster the efficient progression of gene expression. The central dogma of molecular biology holds that genetic information normally flows from DNA to RNA to protein. As a consequence, it has been assumed that genes generally encode proteins and that proteins fulfill not only structural and catalytic functions, but also most regulatory functions in cells. However, early studies demonstrated that RNA can execute functions without being translated into protein. Such RNAs include housekeeping RNAs (ribosomal RNA, transfer RNA, and uridine-rich small nuclear RNAs and small nucleolar RNAs [snRNAs and snoRNAs, respectively]), regulatory RNAs (small interfering RNAs that participate in the RNAi pathway, microRNAs that function primarily as modulators of mRNA translation and stability, naturally occurring antisense transcripts and X-inactive-specific transcript [XIST] associated with the inactive X chromosome in female mammals), and catalytic RNAs (telomerase RNA and ribozymes).

Earlier studies from our laboratory have identified a nuclear population of poly(A)$^+$ RNA that is not chased into the cytoplasm upon termination of transcription. We have been interested in identifying and characterizing the RNA species in this nuclear population of RNA. Because these RNAs, for the most part, are not diffusely distributed throughout the nucleoplasm, we were also interested in identifying the nuclear domains to which they are localized.

We biochemically purified interchromatin granule clusters and associated nuclear domains from mouse liver nuclei, and constructed a cDNA library from poly(A)$^+$ RNA. Clones were sequenced and those that did not show significant similarity to annotated protein-coding genes were further characterized by RNA fluorescence in situ hybridization (RNA-FISH) in various mouse cell lines. Our screen was to identify RNAs that presented interesting localization patterns that were different from what would be expected for protein-coding transcripts. One of the identified cDNA clones localized in a micropunctate nuclear distribution, and in a population of cells, it also localized to a subnuclear domain referred to as paraspeckles. Northern blot and genomic analysis revealed that this clone encodes a ~9-kb nuclear-retained poly(A)$^+$ RNA, transcribed from the *mouse cationic amino acid transporter 2* (*mCAT2*) gene, hence named CAT2 transcribed nuclear-RNA (CTN-RNA). The *mCAT2* gene also encodes the protein coding *mCAT2* mRNA. Both of these transcripts share all protein-coding exons (exons 2–11) and all introns are properly spliced. However, CTN-RNA is transcribed from promoter A and utilizes the distal most poly(A) site, whereas *mCAT2* mRNA is transcribed predominantly from promoters D and E, and utilizes the proximal poly(A) site. CTN-RNA shows tissue-specific expression; elevated levels are observed in liver, brain, and lung. Interestingly, CTN-RNA exhibits adenosine-to-inosine editing (A to I) in its 3′ UTR (untranslated region), which is likely involved in its nuclear retention. Knockdown of CTN-RNA using antisense oligonucleotides designed from the 5′ UTR or 3′ UTR of CTN-RNA, which do not overlap with *mCAT2* mRNA,

also resulted in the knockdown of *mCAT2* transcripts, suggesting that the nuclear-retained CTN-RNA positively regulates the levels of the protein-coding *mCAT2* mRNA. This regulation may occur through the sequestration of destabilizing factors by CTN-RNA. Since *mCAT2* mRNA and CTN-RNA share all protein-coding exons, they could compete for binding of such factors. Therefore, the knock down of CTN-RNA may allow access of these factors to the *mCAT2* transcripts, resulting in their degradation. These data suggest an interesting paradigm whereby a nuclear-retained RNA regulates the level of a protein-coding mRNA.

The cellular function of the mCAT2 protein provides some insight into why its mRNA may be highly regulated. The mCAT2 protein is a cell surface receptor that is a member of the cationic amino acid transporter family of proteins involved in the uptake of extracellular L-arginine, which is essential for sustained NO (nitric oxide) production via nitric oxide synthase 2 (NOS2 or iNOS). However, overproduction of NO has been shown to have a pivotal role in inflammatory responses. Persistent activation of iNOS can lead to the production of toxic levels of NO, resulting in several diseases including multiple sclerosis, HIV-induced dementia, Huntington's disease, Parkinson's disease, stroke, and amyotrophic lateral sclerosis. This highlights the importance of the cellular uptake of L-arginine as a method of regulating inducible NO biosynthesis. Furthermore, a lack of L-arginine in cells causes the production of superoxide via the iNOS pathway resulting in cell death. Because the NO pathway is induced in cells under various stress conditions including viral infection and wound healing as a part of a cellular defense mechanism, it may have evolved a "rapid response" mechanism for regulating the level of *mCAT2* mRNA in cells.

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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 bovine papillomavirus type 1 (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 lifecycle, 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. 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 in DNA replication 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.

ASSEMBLY OF A DOUBLE HEXAMERIC HELICASE

Initiation of DNA replication requires binding of the initiator to the ori, initial opening of the DNA duplex (melting), as well as the assembly and loading of the E1 replicative helicase at the replication fork. For many years, it has been possible to reproduce the sum of these activities in vitro in assays such as the form-U assay, and notably in cell-free DNA replication systems. Since the E1 protein clearly is capable of providing all of the activities required for initiation and carry out these functions in sequence, proteins such as E1 have held great promise as a means to achieve a precise molecular understanding of how DNA replication is initiated in a system of low complexity. In addition, since the end product is a double hexameric helicase, information about E1 assembly would clarify how such enzymes are formed. Currently, it is not known how double hexameric helicases are assembled, how they engage their substrates, or how they processively unwind double-stranded DNA, although many different models have been proposed. A contributing reason for the limited understanding is the uncertainty about what constitutes a bona fide substrate for a replicative DNA helicase. Most helicases have therefore been characterized on artificial model substrates of uncertain relevance. A great advantage of the viral initiator proteins is that they, in the process of assembly, generate the substrate for the helicase, obviating the need for artificial substrates.

Unfortunately, although the E1 double hexamer has long been recognized as the replicative DNA helicase, and therefore the end product of the assembly

process, only limited information about steps preceding double hexamer formation exists. Consequently, how and when in the assembly process melting of the template occurs, whether specific precursors are required for double hexamer formation, and what role nucleotide binding and hydrolysis have, are all unknown. The electrophoretic mobility-shift assay (EMSA) has been a particularly powerful tool for understanding how protein complexes form on DNA, and we have therefore in the past year developed EMSAs to analyze the E1 complexes that form on the viral origin of DNA replication. By using very precisely defined conditions, we have been able to recapitulate the sequence of events that are required for the assembly of the double hexameric helicase. The E1 protein binds initially as a dimer. Upon ATP binding, E1 forms a novel double trimeric complex. Formation of this complex depends on cooperation of two different DNA-binding activities, present in the DNA-binding domain (DBD) and the helicase domain, respectively. ATP binding, but not hydrolysis, is required for formation of the double trimer. The double trimer melts the DNA, providing the first example of a defined complex capable of melting double-stranded DNA. Hydrolysis of the bound ATP triggers formation of a double hexamer, using the double trimer as a precursor. The double hexamer has the capacity to unwind the partially melted DNA in an ATP-dependent manner, providing the opening required for initiation of DNA synthesis.

MUTATIONAL ANALYSIS OF THE E1 DBD SURFACE

The DBD of the E1 protein clearly is responsible for the highly site-specific DNA binding that the E1 protein is capable of, which in turn is responsible for recognition of the origin of DNA replication in the viral genome. Furthermore, on the basis of both structural and biochemical studies of the E1 DBD, the DBD also provides the dimerization surface for the E1 protein and also contains an interaction surface for the DBD from the E2 protein. In addition, based on imaging analysis of, for example, SV40 T antigen, it is likely that the E1 DBD has an active part in the formation of large complexes such as the double hexamer, which is the replicative DNA helicase. To identify additional functions in the DBD, we have performed a complete surface mutagenesis of the E1 DBD. Based on the high-resolution structure of the E1 DBD, we have substituted approximately 70 surface residues for alanine, avoiding areas known from the structural analysis to be involved in DNA binding and dimerization. After screening these E1 mutants for expression and for in vivo DNA replication activity, we were able to identify 18 mutants with defects in DNA replication. Six of these were defective for interaction with E2, and the remaining 12 mutants showed unimpaired interaction with E2 and consequently were defective for DNA replication for other reasons. To determine what biochemical functions might be defective in these mutants, we expressed and purified the 12 mutant E1 proteins and tested them in five different in vitro assays related to DNA replication. None of the mutants showed a defect in either the ATPase or the nonspecific DNA helicase activities, indicating that the protein structure was not greatly affected by the mutations and that most likely, the ability to form hexameric structures is unaffected by the mutations. However, all 12 mutants had severe defects for in vitro DNA replication as well as defects in melting activity and in fragment unwinding activity. By using our recently developed EMSAs, we have been able to test these mutants directly for the formation of double trimers and double hexamers. Of the 12 mutants, 5 had defects in double hexamer formation, indicating that the DBD has an active part in the assembly of the double hexamer. Interestingly, however, mutations that affect dimerization by the E1 DBD are not severely defective for double hexamer formation, indicating that the double hexamer is unlikely held together by this dimer interaction.

DNA REPLICATION AND CHROMATIN INHERITANCE

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Our research focuses on the inheritance of chromosomes and chromatin structures in eukaryotic cells. The primary interest is how DNA replication occurs, how it is regulated, and how it is coordinated with other events such as chromosome segregation during mitosis. Most of our research now focuses on the mechanism of initiation of the process that duplicates the genome which encodes our genetic information. The focus of this research is the origin recognition complex (ORC) which binds to DNA sequences that localize where DNA replication begins along chromosomes. ORC binds a number of proteins during the cell division cycle, and together, they coordinate the chromosome duplication process.

Our previous studies 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. During the past year, we have investigated this reaction in more detail and have identified a new regulatory role for origin DNA sequences in controlling ORC function. 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 bp in the origin DNA and protects them from digestion in vitro by deoxyribonuclease I (DNase I). Upon addition of Cdc6, and only in the presence of ATP, the DNase I footprint is greatly extended to about 85 bp, 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 ORC is required for formation of the extended footprint over the origin DNA since mutant versions of ORC or 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. 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.

What is interesting about these reactions at the origin of DNA replication is the dual role of specific DNA sequences in the control of events prior to the initiation of DNA replication. Stephen Bell's laboratory (MIT) has shown that when ORC is bound to the origin of DNA replication, the ORC ATPase activity is suppressed. We have now shown that when Cdc6 is added to the origin, the ORC ATPase activity is activated and the ATPase requires specific DNA sequences in the origin, particularly the B1 and B2 elements in the origin. These two DNA elements are the region of the origin where the extended footprint occurs. Thus, in one circumstance, ORC ATPase is suppressed by the origin DNA sequences, and later, when Cdc6 binds, ORC ATPase activity is activated by the same origin sequences. We think that this ATPase switch is required to load the minichromosome maintenance (MCM) proteins onto the origin to form the prereplicative complex (pre-RC) that renders the origin competent for subsequent initiation of DNA replication in the S phase of the cell cycle. These results also imply that ORC ATPase activity is cell-cycle-regulated since Cdc6 is only present in yeast cells from mitosis until the cell commits to a new round of cell division.

In human cells, the regulation of ORC is more complicated. We have demonstrated that ORC is not a stable complex in the cell division cycle, but that different complexes exist at different times. Nevertheless, just like in yeast cells, continually proliferating human cells form the pre-RC as they exit mitosis in a process that requires ORC, Cdc6, Cdt1p, and the MCM proteins. In collaboration with Steve Reed's laboratory (Scripps Research Institute), we investigated the regulation of MCM loading onto chromatin by cyclin E.

Cyclin E is often overexpressed in human cancer cells and patients with such tumors, particularly those with breast carcinomas who have a poor outcome for survival. Steve Reed's laboratory has shown that cells that overexpress cyclin E accumulate genomic abnormalities that could cause additional mutations in the

DNA and accelerate cancer progression. In collaboration with his laboratory, we have demonstrated that overexpression of cyclin E causes a reduction in the loading of Mcm4 protein, one of the key pre-RC components. As a result, such cells progress through the G_1 phase of the cell cycle with fewer pre-RCs and this in turn causes slow progression through S phase. We have suggested that progression through S phase with a low number of competent origins of DNA replication may cause some cells to enter mitosis without completing DNA replication, resulting in chromosomal abnormalities.

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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, two graduate students, Aftabul Haque and Guang Lin, from the MCB Program at Stony Brook carried out rotation projects and have joined the lab to conduct their Ph.D. studies.

THE COMPOSITION AND DIVERSITY OF THE PTP FAMILY

Since the human genome sequence became available, one emphasis of the lab has been to define the composition of the PTP family. We have now cataloged the human PTP genes and more recently 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 two broad categories: the classical, pTyr-specific phosphatases and the dual specificity phosphatases (DSPs). The DSPs are operationally defined as members of the PTP family that have the capability of dephosphorylating both pTyr and pSer/pThr residues in vitro. Nonetheless, in terms of physiological function, the "DSPs" may actually show preference for either Tyr or Ser/Thr residues. Some DSPs even target nonprotein substrates such as inositol pholipids and RNA. This study was initiated as a collaboration with Dr. Niels Peter Moller and his colleagues at Novo Nordisk in which we determined that the total number of classical PTP genes in humans is 38. We have found direct orthologs of each of these human PTP genes in the mouse genome. In contrast, we have also discovered 12 PTP pseudogenes that are unique to humans. Interestingly, some of these pseudogenes yield transcripts; however, they would only produce truncated/mutated proteins, the function of which, if any, is unclear. We have continued these genomic analyses independently, most recently publishing a "Practical Guide to Bioinformatics and Data Resources for Computational Analysis of PTPs" in which we define the classical PTPs in a variety of organisms. Furthermore, we have extended this study, in collaboration with Ravi Sachidanandam and his colleagues here at CSHL, to search the human genome sequence for DSPs that are related to VH1, the prototypic DSP, a 20-kD protein that is a virulence factor of *Vaccinia* virus. This proved to be a greater challenge than our similar analysis of the classical PTPs because, although they contain the active site signature motif, the DSPs are less well-conserved overall and therefore harder to identify on the basis of their sequence than the classical PTPs. We have identified 43 human VH1-like DPSs, all but one of which are also found in mouse. Similar searches have been performed in various other genome sequences, including *Drosophilia melanogaster*, *Caenorhabditis elegans*, *Fugu rubripes*, and *Arabidopsis thaliana*, to shed light on the evolutionary history of the PTP superfamily. These data are currently being prepared for publication. In summary, the PTP family in humans is encoded by 106 genes as follows: 38 classical PTPs, 43 VH1-like DSPs, and, from other published studies, 3 cdc25s, 16 mytobularin-like phosphatases, 1 low-molecular-weight PTP, 2 inositol-4-phosphatases, and 3 Sac domain inositol 5-

phosphatases. In comparison, there are 90 human PTK genes, illustrating comparable levels of complexity between the two families. However, it is important to remember that this reflects the minimal level of complexity, with diversity being enhanced by such mechanisms as alternative mRNA splicing, alternative promoter usage, proteolytic processing, etc. Nonetheless, this provides a foundation for future studies aimed at profiling PTP sequences at the protein level.

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 neighbor 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.

RECEPTOR PTPμ AND ENDOTHELIAL CELL FUNCTION

PTPμ is a receptor PTP, the extracellular segment of which consists of one Ig (immunoglobin) domain, four FN III (fibronectin type III) repeats, and a MAM (Mu, A5 and Meprin homology) domain, the latter being a conserved domain unique to PTP μ, κ, ρ, λ, and some cell adhesion molecules. Previously, we had shown that PTPμ is a homophilic adhesion protein, such that the ligand for one molecule of PTPμ is the extracellular segment of another PTPμ molecule expressed on the surface of an apposing cell. Furthermore, we identified the Ig domain as being necessary and sufficient for binding. We have also demonstrated that PTPμ interacts with cadherin-catenin cell adhesion complexes and may regulate the adhesive properties of classical cadherins by dephosphorylating cell junction proteins. PTPμ is expressed predominantly in endothelial cells. In subconfluent cell cultures, PTPμ resides in an intracellular membrane pool; however, as culture density increases and cell contacts form, the phosphatase is localized to sites of cell-cell contact and its expression level increases. These characteristics of PTPμ, which are consistent with a role in cell-cell adhesion, suggest that control of subcellular localization and protein level are important mechanisms to regulate the function of this phosphatase. Furthermore, we noted that overexpression of PTPμ in a bovine aortic endothelial cell line, GM7372, reduced cell number in culture. These effects were most pronounced when cell density was low, the condition that coincides with low levels of endogenous PTPμ, and resulted from increased apoptosis, rather than decreased proliferation. To gain a better understanding of the regulation of PTPμ function, we explored further a structure-function analysis of the enzyme, focusing on the extracellular segment, in particular the importance of the conserved Ig domain, containing the homophilic binding site. We observed that the deletion of the Ig domain greatly reduced the localization of PTPμ to cell-cell contacts and resulted in dispersal of the mutant protein across the cell surface. Similar results were observed in endothelial and epithelial cells, suggesting that the localization of the enzyme was a property of the PTP itself and not a phenomenon unique to particular cells. In addition, deletion of the Ig domain affected localization of PTPμ in subconfluent cells, when homophilic binding to another PTPμ molecule on an apposing cell is not possible, leading to an accumulation of the PTP at the cell periphery in the region occupied by focal adhesions. This apparent localization coincided with changes in the morphology of focal adhesions, as well as in the distribution of cytoskeletal components such as vinculin, paxillin, and actin. These effects were accompanied by increased apoptosis; in fact, deletion of the Ig domain enhanced the apoptotic effect of PTPμ to the highest levels seen with any of the mutant constructs tested. This study therefore illustrates the critical role of the Ig domain in regulation of the localization of PTPμ and the importance of such control for the maintenance of normal cell physiology.

The vascular endothelium represents a barrier that regulates paracellular movement of macromolecules into extravascular compartments. This paracellular pathway of macromolecular flux is controlled in part by tyrosine phosphorylation of adherens junction (AJ) proteins, which integrate cell-cell contact and actin cytoskeleton. Enhanced tyrosine phosphorylation is associated with increased *trans*-endothelial flux (i.e., decreased barrier function). Several AJ proteins are known to be tyrosine-phosphorylated, such as VE-cadherin and catenins, but the mechanistic links between tyrosine phosphorylation and barrier function remain to be defined. In collaboration with Dr. Simeon

Goldblum (University of Maryland) we have been exploring potential roles for PTPμ in regulating AJ tyrosine phosphorylation and barrier function in pulmonary vascular endothelial cells. We observed that the PTPμ was expressed in postconfluent human pulmonary artery and lung microvascular endothelial cells (HMECs), where it was almost exclusively restricted to cell-cell boundaries. Ablation of PTPμ expression by RNA interference dramatically impaired barrier function. Conversely, overexpression of wild-type PTPμ enhanced barrier function. These data are consistent with PTPμ exerting a regulatory influence over barrier function. We have been trying to identify physiological substrates of PTPμ to explore the mechanisms underlying these effects. In light of its association with classical cadherins, we tested whether there was an interaction between PTPμ and VE-cadherin. We observed direct binding between the recombinant proteins in vitro and colocalization and coimmunoprecipitation of PTPμ and VE-cadherin from HMECs. Interestingly, overexpression of PTPμ in HMECs led to a decrease in tyrosine phosphorylation of VE-cadherin, with specificity indicated by the fact that there were no changes in tyrosine phosphorylation of β catenin. Whether this is the critical regulatory event in the control of barrier function remains to be established.

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 dysfunctional Jnk signaling. We have now generated JSP-1 knockout mice, which are born in Mendelian ratios, and we are characterizing their phenotype. Thus far, we have observed that ablation of JSP-1 leads to attenuation of the inflammatory response to Gram-negative bacterial endotoxin, suggesting an important role in the innate immune system. The mechanistic basis for this effect is currently under investigation. 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. Currently, we are 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.

Two Distinct DSPs Encoded in Alternative Open Reading Frames of a Single Gene at Human Chromosone 10q22.2

We identified a gene located on chromosome 10q22.2, which utilizes alternative open reading frames (ORFs) to encode TMDP (testis and skeletal specific DSP) and a novel enzyme that we term MDSP (muscle restricted DSP). This gene organization is conserved in mice. Although this is reminiscent of the p16Ink4/p19Arf locus, to our knowledge, this is the first example of a gene from which two distinct proteins of the same family are expressed using alternative ORFs. We demonstrated that both MDSP and TMDP are restricted to specific tissues, skeletal muscle and testis, respectively. Expression of both proteins was markedly increased at approximately the third week after birth and continued to increase gradually into adulthood, implying that the physiological functions of both DSPs are specific to the mature organs. More recently, we have found that MDSP may be overexpressed in certain tumors, and we are currently focusing on a functional characterization of the enzyme.

DUSP23

The gene for this DSP is located at chromosome 1q22.23, a region that is 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 with 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 (RNAi), 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.

PTP OXIDATION AND THE ROLE OF PTP1B AND TC45 IN INSULIN SIGNALING

One emphasis of the lab remains the characterization of two nontransmembrane PTPs that are now known to exert selective effects on signal transduction in vivo—PTP1B, the prototypic member of the PTP family, and its closest relative, TCPTP. TCPTP exists in two alternatively spliced forms that share the same catalytic domain but differ in the sequence at the carboxyl terminus of their regulatory segments. Like its closest relative PTP1B, TC48 is targeted to the cytoplasmic face of membranes of the endoplasmic reticulum, whereas TC45 is located in the nucleus in the basal state, but translocates to the cytosol in response to stimuli. Although much attention has focused on the role of PTP1B in dephosphorylating the insulin receptor (IR), we have made an exciting breakthrough with the demonstration of a role for TC45 in the regulation of insulin signaling. We have shown that insulin induces production of reactive oxygen species. Using our modified "in-gel" PTP assay, we demonstrated that PTP1B and the 45-kD spliced variant of TCPTP were rapidly and reversibly oxidized and inhibited in response to insulin in Rat 1 fibroblasts. Our hypothesis is that tyrosine-phosphorylation-dependent signaling, induced by stimuli such as insulin, may be fine-tuned by transient oxidation and inhibition of those PTPs that normally function to attenuate the signaling response (Fig. 1). PTP1B is well established as an antagonist in insulin signaling. Using RNAi to ablate TC45 in Rat 1 and HepG2 cells, we demonstrated that small interfering RNA (siRNA) to TC45 enhanced insulin-induced activation of PKB/AKT and led to hyperphosphorylation of the IR β subunit. Using the substrate trapping mutant form of TCPTP (D182A) in HepG2 and 293 cells, we observed that the β subunit of the IR is a

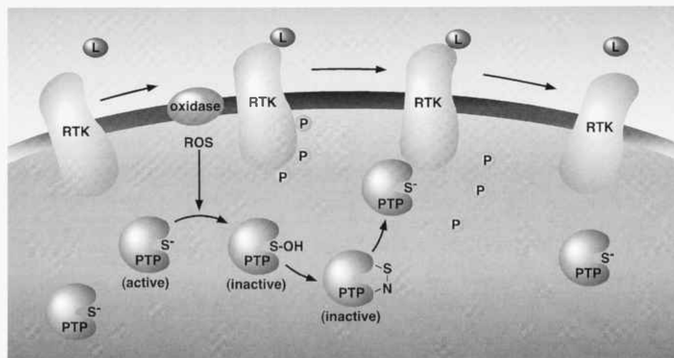

FIGURE 1 Regulation of protein tyrosine phosphatase (PTP) activity by reversible oxidation. Ligand-dependent activation of a receptor protein tyrosine kinase (RTK) triggers the production of reactive oxygen species (ROS). ROS oxidizes the active site Cys residue of members of the PTP family, converting it from a thiolate ion (the active form) to sulfinic acid and then to a cyclic sulfenamide, with concomitant conformational changes at the active site. Oxidation results in inhibition of PTP activity, thereby promoting tyrosine phosphorylation. However, oxidation of the PTPs is transient. Restoration of PTP activity following reduction back to the thiolate form of the active site Cys residue terminates the tyrosine-phosphorylation-dependent signal. A variety of growth factors, hormones, and cytokines induce ROS production and stimulate tyrosine phosphorylation. We are developing methods to identify the PTPs that become oxidized in response to a physiological stimulus as a way of establishing links between particular PTPs and the regulation of defined signaling pathways.

direct substrate of TCPTP. Furthermore, blotting of immunoprecipitates of IR β from siRNA-treated cells with phospho-specific antibodies indicated that TC45 displays preferential recognition of particular sites within the receptor.

We have explored further the effects of PTP1B and TC45 on insulin signaling using fibroblasts (MEFs) derived from PTP1B–/– and TCPTP–/– mice, in collaboration with Tony Tiganis (Monash University, Australia). Ablation of PTP1B resulted in enhanced, insulin-induced phosphorylation of both IR β and IRS-1, in contrast to TCPTP–/– MEFs in which hyperphosphorylation of IR β occurred in the absence of an effect on IRS-1. Furthermore, whereas PKB phosphorylation was elevated/prolonged in cells that lacked either PTP1B or TCPTP, compared to their wild-type or reconstituted counterparts, Erk 1/2 phosphorylation was enhanced only in PTP1B–/– cells. These data suggest that the two PTPs may exert differential effects on insulin signaling. To explore further the mechanism of action of these PTPs, we examined the phosphorylation status of IR β using phospho-specific antibodies to Y1162/63, from the activation loop of the IR PTK, and Y972, a site that contributes to the recruitment of IRS-1. Insulin-induced phosphorylation of both sites was enhanced in the absence of PTP1B; however, the kinetics of phosphorylation were similar in the presence or absence of the PTP, peaking at 2 minutes and declining rapidly thereafter. In contrast, phosphorylation of Y1162/63 was not altered at early time points of insulin stimulation in the absence of TCPTP; instead, phosphorylation was sustained over a prolonged time course out to 100 minutes, compared to TCPTP+/+ cells in which phosphorylation had declined to baseline by this time. In addition, we observed that phosphorylation of Y972 was enhanced in TCPTP–/– MEFs early in the time course of insulin stimulation, when Y1162/63 phosphorylation was not altered significantly, indicating that TCPTP exerts differential, independent effects on these two phosphorylation sites. The effects of these PTPs can be distinguished further using RNAi to suppress TCPTP expression in PTP1B–/– MEFs. In PTP1B–/– MEFs, we observed that insulin-induced phosphorylation of PKB declined at 40–60 minutes, whereas phosphorylation was maintained for at least 100 minutes following RNAi-induced suppression of TCPTP in these cells. In contrast, suppression of TCPTP did not alter the time course of Erk2 activation, illustrating specificity and that the ablation of TCPTP did not produce a general enhancement of signaling. Furthermore, we observed that suppression of TCPTP by RNAi in PTP1B–/– MEFs led to prolonged phosphorylation of Y1162/63 in IR β. These observations illustrate that in a cellular context, PTP1B and TCPTP are not redundant, but rather act in a coordinated manner to exert differential effects in the regulation of IR β phosphorylation and signaling in vivo.

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RAS AND RHO GTPASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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Our research focuses on signal transduction pathways involving members of the Ras and Rho GTPases and the physiological processes that they regulate. Ras and Rho family members have been documented to 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. To understand how alterations in Ras and Rho signaling components contribute to these disease processes, it is essential to comprehend their normal functions at both a molecular and a cellular level. Efforts in my laboratory have focused on defining the role and mechanisms by which Ras and Rho family members exert their effects on specific aspects of tumorigenesis and on neuronal development. Below are highlighted the main projects that have been carried out during the past year.

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 analyses 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 "bone fide" effector of Rap1 in the process of epithelial migration.

More recently, we identified a *Drosophila* Rap1-specific exchange factor, DPDZGef, which is responsible for Rap1 activation in migrating embryonic epithelia. Genetic and biochemical experiments showed that the *DPDZGef* gene is indispensable for epithelial migration in the embryo and that it activates the Rap1/Canoe pathway. Interestingly, the mammalian counterpart of the DPDZGef 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 is conserved across species.

ROLE OF DOK PROTEINS IN LEUKEMIA SUPPRESSION

$p62^{dok}$ (also called Dok-1) was cloned as a major phosphorylation substrate of the $p210^{bcr/abl}$ oncoprotein in CML (chronic myelogenous leukemia) blasts. This protein was termed Dok (downstream of kinases), since it was also found to be a common substrate of many receptor and cytoplasmic tyrosine kinases. Subsequently, four additional Dok family members have been identified. Among them, 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. In last year's Annual Report, we reported that Dok-1 acts as a negative regulator of growth-factor-induced cell proliferation and that it does so at least in part by negatively influencing the Ras/MAPK pathway. We also reported that Dok-1 inactivation causes a significant shortening of the latency of the fatal myeloproliferative disease induced by $p210^{bcr\text{-}abl}$. These findings

implicated a role for Dok-1 in the negative regulation of p210$^{bcr\text{-}abl}$ signaling and leukemogenesis.

Dok-1 (or *Dok-2*) single knockout (KO) mice did not show gross aberrant phenotypes and displayed normal steady-state hematopoiesis. This observation suggested that the two proteins could exert redundant function(s). In collaboration with Dr. Pandolfi's group (Memorial Sloan-Kettering Cancer Center), we recently characterized *Dok-1/Dok-2* double KO (DKO) mice. Concomitant *Dok-1* and *Dok-2* inactivation resulted in aberrant hematopoiesis and Ras/MAP kinase activation. Inactivation of both genes increased the proliferative potential of myeloid bone marrow (BM) cells and protected them from growth-factor-deprivation-induced cell death. DKO BM cells displayed elevated levels of Ras and MAPK upon GM-GSF stimulation. Strikingly, all *Dok-1/Dok-2* DKO mutants spontaneously developed transplantable CML-like myeloproliferative disease, likely resulting from increased cellular proliferation and reduced apoptosis. These findings reveal a critical role for Dok1 and Dok2 in the control of the hematopoietic compartment homeostasis.

REGULATORS OF RHO GTPASES CONTROLLING KEY ASPECTS OF NEURONAL FUNCTION AND DEVELOPMENT

Accumulating data indicate that the Rho family of GTPases and their regulatory molecules have critical roles in many aspects of neuronal development, particularly in determining neuronal morphology via their various actions on the actin cytoskeleton. Given their importance in neuronal processes, it is therefore not surprising that mutations in genes encoding a number of regulators and effectors of the Rho-GTPases are associated with diseases affecting the nervous system. Aberrant Rho signaling has been shown to underlie various forms of mental retardation as well as defects in axon regeneration. We have been focusing on two regulators of the Rho GTPases, oligophrenin-1 and DOCK7, for which we demonstrated roles in dendritic morphogenesis and axon formation, respectively.

Oligophrenin-1 (*OPHN1*) codes for a protein that has a Rho-GAP domain shown to negatively regulate Rho family members. It was originally identified by the analysis of a balanced translocation t(X;12) observed in a female patient with mental retardation (MR). Subsequently, a mutation within the *OPHN1*-coding sequence was identified in a family with non-syndromic X-linked MR (MRX 60). In both cases, *OPHN1* mutations were associated with a loss of, or dramatic reduction in, mRNA product. More recent studies have also reported the presence of *OPHN1* mutations in families with MR associated with epilepsy and/or cerebellar hypoplasia. Until recently, nothing was known about the function of *OPHN1* in the brain.

Upon characterizing the localization of OPHN1, we found that the protein is expressed in neurons in major regions of the brain, including the hippocampus, cortex, and cerebellum, and is present in axons, dendrites, and dendritic spines. We obtained evidence for the requirement of OPHN1 in dendritic spine morphogenesis of hippocampal neurons. Using RNA interference and antisense approaches, we showed that knockdown 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 adapter 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 novel Rac-interacting protein we recently identified in a search for regulators of Rac1. Specifically, we conducted yeast two-hybrid (YTH) screens, using a human fetal brain library with the dominant-negative mutant form of Rac1, Rac1N17, as bait. Further analysis showed that DOCK7 specifically associates with and activates Rac GTPases. Blast searches revealed that DOCK7 is a member of the DOCK180-related superfamily of proteins, which emerged as novel activators of the Rho GTPases. Among the DOCK180 family members, DOCK180 and DOCK2 have been most extensively studied. DOCK180 has crucial roles as upstream activators of Rac in cytoskeletal remodeling, cell migration, and phagocytosis, and DOCK2, in lymphocyte homing to lymphoid tissues.

We began the characterization of DOCK7 by determining its presence, distribution, and subcellular

localization in the brain. We found that DOCK7 is highly expressed in major regions of the brain during early stages of development. Moreover, we observed that in hippocampal neurons, DOCK7 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. Ectopic expression of DOCK7 resulted in the formation of multiple axons, whereas knock down of DOCK7 protein levels prevented axon formation. Our preliminary data support a model in which a DOCK7 protein complex triggers local Rac activation resulting in altered cytoskeletal dynamics, which is key for axon formation and elongation. Together, our studies contribute to defining the molecular mechanisms by which neurons acquire their polarity, which is crucial for nearly every aspect of neuronal function. These studies may also shed light on axon regeneration processes.

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Ronnie Packer, lab tech, Linda Van Aelst's lab.

NEUROSCIENCE

By using a wide variety of powerful approaches, CSHL neuroscientists are making extraordinary progress in understanding nervous system structure and function on many levels—from genes, to proteins, to synapses, to the vast networks of brain neurons that ultimately make us who we are.

Several scientists here are using the fruit fly *Drosophila* to systematically study various neurological processes, most—if not all—of which also occur in humans or are relevant to human biology or disease. Yi Zhong is using the power of *Drosophila* molecular genetics to study the pathology of human Alzheimer's disease. Last year, he found that producing the human amyloid-β peptide Aβ42 in the fruit fly brain was sufficient to cause nerve cell death, "plaque" formation, and age-dependent memory loss reminiscent of Alzheimer's disease. Zhong is now using these "Alzheimer's flies" to explore the mechanisms underlying Aβ42-induced age-dependent memory loss and neurodegeneration.

Tim Tully's group has recently obtained evidence from their *Drosophila* studies that confirms a previously controversial role for proteins called NMDA (*N*-methyl-D-aspartate) receptors in Pavlovian learning and long-term memory formation. Because NMDA receptors function in the brains of both flies and humans, Tully's findings reveal a fundamental, evolutionarily conserved role for these receptors in vertebrate and invertebrate learning and memory.

By studying a *Drosophila* memory mutant called *radish*, Josh Dubnau has uncovered the first clues about the molecular and anatomical basis of a form of long-lasting memory—also present in humans—known as anesthesia-resistant memory. Dubnau's group found that the *radish* gene encodes a particular enzyme (phospholipase-A2), and that the enzyme is present in a novel anatomical region of the fly brain not previously associated with learning and memory. These discoveries enable the researchers to explore many new aspects of the molecular and cellular basis of anesthesia-resistant memory.

Using rodents, Karel Svoboda focuses on understanding the mechanism of memory formation. He has developed laser-based tools that can rapidly measure changing neural circuits. Svoboda's group has discovered that neural networks in the mammalian cortex (the portion of the brain that processes sensory experience) wire up in a highly directed and precise fashion in response to experience. These findings suggest that cortical neural circuits respond to sensory experience in a tightly controlled and predictable pattern.

Josh Huang studies how the brain forms neural networks that respond to GABA, the primary inhibitory neurotransmitter or "off switch" for neurons. His research revealed that GABA synapses in the visual cortex (the part of the brain that processes visual signals) develop "automatically" by genetically encoded mechanisms, without sensory input. Subsequently, Huang's group found that after the basic GABA synapses are in place, they undergo a long period of maturation that *is* shaped by sensory experience. Part of Huang's ongoing research is uncovering the molecular mechanisms that allow GABA synapses to change according to experience.

A complete understanding of brain function requires the comprehensive mapping of the brain's microcircuitry. In other words, the detailed three-dimensional geometry of individual neurons must be fully documented, as must the patterns of connections between the neurons and the strengths of those connections. Dmitri Chklovskii has discovered strongly preferred patterns of connectivity or "scaffolds" within the wiring diagram of the rat brain. The patterns are likely to correspond to modules that have an important role in brain function not only in rats, but also in humans.

Alexei Koulakov has developed a theoretical model of the visual system that predicts how axons (nerve cell projections that propagate signals) can work in coordinated groups. The model approximates the relative strengths of the connections between axons and their targets. The model fits in vivo experimental observation and makes testable predictions about neural network structure and function.

Chklovskii and Koulakov have also collaborated to develop a method for mapping brain microcircuitry. They created software that automatically aligns images of successive slices of brain tissue captured by electron microscopy. This state-of-the-art software generates three-dimensional views of the

complete microcircuitry of the region analyzed.

Holly Cline studies the development of neural connections in the brain and spinal cord in tadpoles, which have the advantage of being transparent, allowing Cline and her colleagues to watch the nervous system develop using time-lapse microscopy. Combining this imaging approach with gene transfer methods led Cline's group to the discovery that a protein called CPG15 controls the development of synaptic connections between motor neurons in the spinal cord and muscles. Cline had previously shown that this same protein performs a similar function in the brain. Her continuing work will examine these and other key aspects of brain development and plasticity.

Grigori Enikolopov studies the molecular events that guide the generation of new neurons in the brain. In particular, he is interested in the role of the versatile signaling molecule nitric oxide (NO), which guides stem cells as they develop into mature neurons. Using *Drosophila*, amphibians, and mice as model systems, Enikolopov and his colleagues have examined how NO controls neurogenesis during development and in adulthood. In other preclinical studies, they are manipulating NO signals to expand bone marrow hematopoietic stem cells for medical applications. The researchers are also studying the effects of therapeutic drugs on the generation of new neurons in the adult brain with the goal of coaxing stem cells to replace neurons that die due to neurodegenerative disorders such as Parkinson's disease.

Lee Henry is working to understand the unique processes that control how taste buds develop. By developing pioneering methods for analyzing the minute quantities of genetic instructions (messenger RNA) produced by single cells, he is profiling how changes in gene expression patterns enable progenitor cells to develop into taste receptor cells. These changes in gene expression patterns are providing valuable clues concerning the development, structure, and function of taste buds.

Anthony Zador is one of a number of CSHL scientists who are exploring the unique computational style the brain uses to solve hard computations that remain far beyond the reach of the fastest digital computers. Zador is approaching this problem by studying "selective auditory attention" or the ability of the brain to focus its conscious awareness on particular sounds. Most of what is known about the auditory cortex (the part of the brain that interprets sound) comes from studies of anesthetized animals. To understand selective auditory attention, however, the cortex of unanesthetized animals must be studied. Zador has developed methods for studying the activity of neurons in the auditory cortex of awake rodents. His group has found that the diversity of neural responses in awake rodents is much greater than that in anesthetized rodents, and they are currently elucidating the causes of this diversity.

In other work this year, Zador collaborated with Roberto Malinow in a study of a form of associative, Pavlovian learning known as fear conditioning. By using a recombinant version of AMPA (α-amino-3-hydroxy-5-methyl-4-isoazole) receptors that specifically tags newly strengthened synapses, the researchers found that up to one third of the neurons in the amygdala strengthen their synapses in response to fear conditioning. This indicates that rather than being restricted to a small subset of neurons, long-term memories are widely distributed among a large proportion of neurons. Malinow and Zador also discovered that blocking synapse strengthening in as few as 10–20% of the relevant neurons was sufficient to impair long-term memory formation. This finding contradicts the conventional view that widely distributed memories are tolerant to perturbation and will transform the accepted wisdom in the field.

Short-term or "working" memory is an important process that enables us to interact in meaningful ways with others and to comprehend the world around us on a moment-to-moment basis. Working memory is believed to be fundamental to many cognitive processes including reading, writing, holding a conversation, playing or listening to music, decision making, and thinking rationally in a general sense. Carlos Brody's group is exploring how neurons interact with one another to form neural networks that underlie working memory. Based on behavioral studies of decision making and mathematical modeling with "spiking neurons," the researchers have discovered a surprisingly simple network architecture that supports all three neurological phases of decision-making behavior (loading phase→memory phase→decision phase). The model is a significant advance toward understanding a fundamental property of brain function.

Zach Mainen is combining behavioral studies, recordings of neuronal microcircuit activity, and computational methods to explore aspects of learning, goal-directed behavior, and pathologies including schizophrenia, obsessive-compulsive disorder, and Parkinson's disease. By studying the activity of ensembles of brain neurons in awake behaving rats, Mainen's group has uncovered clues about how brain circuits normally function to process information, as well as how they may malfunction in the aforementioned pathologies.

Partha Mitra combines theoretical, experimental, and computational approaches to understanding brain function. In one study, Mitra is exploring how birds learn to sing as a means of uncovering principles that govern brain development, learning, and memory across the board, including in humans. By recording and analyzing all of the vocalizations that young zebra finches make as they learn to sing, Mitra and his colleagues have discovered that when the birds awaken from sleep, they are dramatically *worse* singers than they were the previous day. In addition, individual birds who are the least tuneful during their "morning rehearsals" eventually become the best singers of all. More work is required to explain this "one step back, two steps forward" effect of sleep on the brain circuits that consolidate vocal learning. For now, a useful analogy is the tempering of steel, in which to gain its ultimate structure and strength, the metal is first weakened. Vocal learning in songbirds bears similarities to human speech acquisition: Novice birds go through a period of "screeching" before learning to imitate songs accurately, much as babies babble before grasping words. Therefore, the new research highlights the need for a quantitative study of the effects of sleep on learning in humans.

Stephanie Dennison, lab tech, Josh Dubnau's lab.

COMPUTATIONAL SYSTEMS

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

Our lab is interested in how neurons interact with each other to form networks that underlie 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. During 2004, Carlos Brody (PI) and Christian Machens (postdoc) completed a theoretical model for understanding how properties of neuronal responses can be flexibly modulated. We devised practical, experimentally feasible tests of the model and further generalized the theoretical framework to a variety of architectures of networks of neurons. Amandine Penel (postdoc) and Chris Hollweg (technician) used a new psychophysical reporting procedure to assess perception of temporal intervals. The procedure was successfully validated by testing, and confirming, a surprising set of predictions that followed from applying a signal detection analysis to the procedure's results. Santanu Chakraborty (Watson School graduate student) continued his collaboration with David Tank (Princeton), studying network properties of neurons in the goldfish brain that support the short-term memory of eye positions. He found slow oscillating patterns in these neurons' responses and devised a simple model that accounts for the unexpected presence of these oscillations. Carlos Brody provided assistance to Sandra Kuhlman (postdoc, Huang laboratory, CSHL) in the development of a model that describes interactions between inhibitory mechanisms and synaptic plasticity rules during ocular dominance column formation.

A Robust Neural Model of Two-stimulus-interval Discrimination

C. Machens, C. Brody

In our daily lives, our minds can flit from thought to thought with remarkable speed and flexibility. A simplified task that requires rapid shifts between different mental actions is known as two-stimulus-interval discrimination. Subjects must first perceive a brief stimulus (f1), maintain it in working memory for several seconds, and then compare it with a brief second stimulus (f2) to immediately decide which of the two stimuli was larger. On the basis of electrophysiological data from awake behaving monkeys, recorded by Ranulfo Romo (UNAM, Mexico), we developed a simple model, based on two mutually inhibitory populations of neurons, that captures the three phases of the task within a single framework. This model required a precise tuning of its synaptic parameters.

In a further exploration of the framework underlying the model, we are now using ideas regarding robust short-term memory that were put forth by Alex Koulakov and Adam Kepecs here at CSHL, among others, to now make the neurons of our previous model both heterogeneous and hysteretic. This has led to a spiking neural network model of sequential discrimination that (1) is more robust against changes in its synaptic parameters and (2) displays a larger variety of behaviors across different neurons, in comparison to our original model. Surprisingly, this variety of behaviors reproduces much (but not all) of the variety found in experimental data recorded during a sequential discrimination task.

Human Time Perception in Sequences

A. Penel, C. Hollweg

We have developed a new psychophysical task for the investigation of time perception in sequences. It involves participants' spatial translation of their temporal representation: After hearing a sequence of three sounds, they have to position a vertical line within a horizontal bar representing the sequence's first and last sounds. With sequences that are 1 second long and a second tone distributed uniformly within this interval, pilot data had shown a categorization of responses to two 500-msec intervals when the second tone was presented within a range centered around 500 msec after the first sound. An experiment conducted

with sequences 1 second and 1.2 seconds confirmed this categorization to an equal subdivision of the sequence when the second tone is presented around the midpoint.

From the data obtained, predictions could be generated for a classical psychophysical task. Data confirming these predictions were a necessary condition for the validation of the new "spatial translation of temporal representation" method. More precisely, in a task in which participants had to compare two sequences presented successively (say whether in the second sequence, compared to the first, the middle sound was played closer to the first or to the last sound), heightened sensitivity was predicted at the categorization zone's boundaries. A second experiment confirmed these predictions. This work shows that the perception of a time interval in a sequence is fundamentally different from that of the same interval presented in isolation. It provides the first evidence for temporal categorical perception and shows that the human brain favors a temporally regular (isochrone) representation of sequences.

Network Interactions in the Goldfish Oculomotor System

S. Chakraborty [in collaboration with David Tank, Princeton]

We are studying the underlying architecture of a system of neurons in the goldfish called the oculomotor integrator. Understanding the mechanism by which neurons in this system generate persistent firing in response to a transient burst of input could be important in understanding short-term memory-like behavior in many other systems.

We have analyzed the firing rate data from pairs of oculomotor neurons and combined it with modeling to predict the nature of synapses involved in connecting them. We also see evidence of underlying oscillations in the firing of these neurons despite being persistent on average. Currently, we are building models to understand what kinds of underlying neural circuits could give rise to the observed oscillations in an effort to predict the circuits that are responsible for persistent activity in the goldfish.

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PRINCIPLES OF BRAIN DESIGN

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

Full understanding of the brain seems impossible without a comprehensive account of neuronal circuits. As the pattern of synapses between neurons underlies information processing in neuronal circuits, we must describe this pattern and discover the basic principles underlying synaptic connectivity. As the shape of a single neuron affects transformations performed on its inputs, for example, through its electrotonic properties, we must describe neuronal shapes and understand their significance. Full understanding of development also requires a comprehensive description of neuronal shape and synaptic connectivity; they are, after all, the objective of development. We focus on understanding synaptic connectivity and neuronal shape with the goal of discovering basic principles of brain design and function. We pursue this goal on several levels through developing automated image processing algorithms and creative data analysis and building a theoretical framework based on engineering principles.

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 *Caenorhabditis 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, Albert Einstein College of Medicine). 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 the 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, CSHL). 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 for 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). 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 EMs 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 (Fig. 1).

Development of the automated reconstruction algorithms follows a hybrid approach. We aim to maximally automate reconstruction while retaining manual control over the process, using convenient monitoring and editing tools. Eventually, experience gained with our manual tools will be incorporated into the automatic algorithm. We expect that fully reconstructing a nervous system of an organism, such as *Drosophila*, will make an impact on neurobiology comparable to that of sequencing DNA on molecular biology.

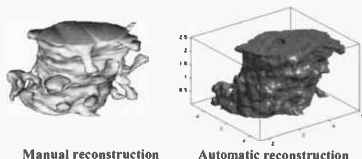

FIGURE 1 Dendrite segment reconstructed by our automatic algorithm compared with the manual reconstruction by the experts (J. Fiala and K. Harris, http://synapses.mcg.edu). Although the general quality of automatic reconstruction is reasonable, some discrepancies are present. We are developing manual editing tools for online correction of the automatic reconstruction.

STATISTICAL DESCRIPTION OF SYNAPTIC CONNECTIVITY

Although a complete description of neuronal circuits can only be achieved with EM, there are several reasons other approaches must be pursued in parallel. First, the complete reconstruction of a complex circuit such as the mammalian cortical column is many years away, yet demand already exists (among experimentalists and theorists) for an approximate description of circuitry. Second, synaptic connectivity varies among animals and within one animal over time and EM cannot be applied in vivo and on a large enough scale to explore variability. Third, EM is not well suited to detect differential gene expression, an important marker of neuronal class and cell processes. This is why, in parallel with reconstructions from EM serial sections, we analyze light microscopy and electrophysiology data. Such description must necessarily be statistical, or probabilistic, because the presence of a synapse cannot be unequivocally established (in light microscopy) and because only a small subset of neurons can be probed in each experiment, meaning that data from different brains must be somehow combined.

We are assembling geometric connectivity maps using light microscopic 3D reconstructions of axonal and dendritic arbors. This approach is based on the concept of potential synapse, a location in neuropil where an axon and a dendrite come within a certain distance so that a synapse can be formed by growing a spine or a bouton. The exact distance depends on the type of a synapse that can exist. If the dendrite is spiny, the distance is given by the spine length (~2 μm). Otherwise, the distance between the dendritic and axonal centroids is given by the sum of their radii. Using 3D reconstructions of neurons from different cortical layers, we calculate geometric input and output maps. Such maps show the number of potential synapses expected between neurons at given locations. As expected, these maps exhibit layer specificity in potential synaptic connectivity. Yet, the potential connectivity domain is rather wide, a few hundred microns, with scale usually associated with the cortical column. Therefore, potential synaptic connectivity within a cortical column is close to all-to-all. In other words, any two neurons within a cortical column can make a synapse if they need to.

GEOMETRIC CONNECTIVITY MAP IS A USEFUL STARTING POINT FOR DESCRIBING SYNAPTIC CONNECTIVITY

As potential synapse is a necessary condition for an actual synapse, the number of potential synapses provides an upper bound on the number of actual synapses. Geometric connectivity maps are particularly significant in view of the structural plasticity in adult neocortex: Actual synapses can appear and disappear while potential synapses stay put. Therefore, an invariant description of synaptic connectivity should be done on the level of potential synapses.

COMPARISON OF GEOMETRIC CONNECTIVITY MAPS WITH MAPS FROM PHOTOSTIMULATION EXPERIMENTS

Although potential synaptic connectivity provides a convenient description of a neuronal circuit, activity of neurons is still determined by the currents injected at the actual synapses. To understand how potential

synaptic connectivity is related to actual, we are collaborating with the Svoboda laboratory here at CSHL; they use laser-scanning photostimulation to measure the spatial distribution of functional input to individual neurons in L2/3. Photostimulation results were compared with geometrical maps obtained from quantitative morphological reconstructions of labeled neurons. With this approach, we were able to test whether neuronal morphology and the overlap of dendrites and axons predict functional circuits. It turns out that for most individual projections (e.g., between layer 4 and layer 2/3), geometry predicts functional inputs up to a single scale factor: synaptic strength per potential synapse. This factor, however, varies from projection to projection and exceptionally within a projection.

STATISTICAL ANALYSIS OF FUNCTIONAL CONNECTIVITY

Another approach to describe connectivity is by electrophysiologically recording from neuron pairs. Because the number of cells from which recordings can be made is limited, this approach is necessarily based on sampling. Analysis of an exceptionally large data set of recordings (in collaboration with S. Nelson, Brandeis University) has revealed that within a cortical column, connectivity is highly nonuniform. Distribution of connection strength, as measured by the average EPSP amplitude, has a heavy tail. We found that 17% of connections contribute 50% of synaptic weight. In addition, these connections are highly clustered, forming a skeleton of stronger connections in the sea of weaker ones (Fig. 2). This description contrasts strongly with the random or uniform connectivity assumptions made by many computational neuroscientists when analyzing network dynamics.

SEARCH FOR ENGINEERING PRINCIPLES OF BRAIN DESIGN AND FUNCTION

As demonstrated by the *C. elegans* example, a synaptic connectivity matrix (or a statistical description) is not by itself sufficient to understand brain function. Additional experimental data are needed, such as, for example, electrophysiological recordings. However, electrophysiological experiments in *C. elegans* are notoriously difficult, perhaps, explaining why nervous system function has not been understood despite the unprecedented completeness of the anatomical data. Yet the situation is changing quickly, thanks to genetically encodable calcium indicators, which allow monitoring neuron and muscle activity in vivo. We are combining anatomical information with calcium imaging experiments to understand how undulatory locomotion is generated and controlled in *C. elegans* (in collaboration with J. Pierce-Shimomura, University of California, San Francisco, and C. Bargmann, The Rockefeller University). Unlike most computational neuroscientists, who begin modeling neuronal dynamics by assuming a circuit, we have an advantage of knowing the actual circuit. In addition, our theoretical predictions can be tested relatively easily in *C. elegans*, making it an excellent model system.

IN DEVELOPING A THEORETICAL DESCRIPTION OF NEURONAL CIRCUITS, IT IS IMPORTANT TO CHOOSE AN APPROPRIATE LEVEL OF ABSTRACTION

Such choice must rely on our (rather rudimentary) understanding of the basics of circuit function. For example, how much of circuit functionality is described by the connectivity matrix? What is the significance of neuronal layout and shape? How important is the location of synapses on the neuron? Why are some connections implemented with chemical synapses and others, with gap junctions? To help answer these questions, we study neuronal circuits from the engineering point of view, which includes answering *why* questions. As biological systems have evolved over hundreds of millions of years, design tradeoffs are embedded in the blueprint of such systems. It is thus natural that a theoretical approach to biology must incorporate aspects of constrained optimization.

FIGURE 2 Local cortical circuits can be described as a skeleton of stronger connections (*bold*) immersed in the sea of weaker ones (*black*; unidirectionals are *dashed*, bidirectional are *solid*). Strong connections are likely to influence network dynamics disproportionately.

Particular success has been enjoyed by the wiring optimization principle, which is rooted in Cajal's laws of wiring economy. By using this principle, we are able to explain many aspects of brain design, such as neuronal placement, dimensions of axons, dendrites, and the existence of spines, as well as the segregation of the neocortex into the gray and white matter.

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. Solution of such a problem in the allotted volume requires all the major features of neuronal morphology, such as axon and spiny dendrites. Therefore, one need not look further to find a reason for their existence. We are extending this work to understand branching and specific placement of synapses. Comparing optimization predictions with anatomy will help us to understand the functional significance of neuronal shape.

STATISTICAL SEARCH FOR NEURONAL MODULES IN WIRING DIAGRAMS

Another difficulty with using the connectivity matrix is that such representation of the neuronal circuit is too detailed for comprehensive understanding. In an attempt to simplify the description of the neuronal circuit, we search for multineuron modules (smaller than invertebrate ganglia or vertebrate nuclei and cortical columns). The advantage of modular description is illustrated by electrical engineering, where a description of a circuit in terms of operational amplifiers, logical gates, and memory registers is often preferred to that showing each transistor, resistor, and diode. However, unlike electrical engineers who designed these modules themselves, neurobiologists need to discover such modules. Specifically, we search for a few neuron connectivity patterns that are statistically overrepresented in *C. elegans* relative to the expectations from the random matrix ensemble. We performed similar analysis on the neocortical connectivity data, obtained by sampling. Interestingly, we have found that similar patterns, which we call motifs, are overrepresented in both *C. elegans* and the rat neocortex. In particular, reciprocally connected neuronal pairs are four times more common than expected by chance. Several triplet connectivity patterns are overrepresented in both *C. elegans* and the cortex (Fig. 3). A notable exception is the feedback loop (#11), which is overrepresented only in the neocortex. In the future, we plan to determine the significance of these motifs for circuit function.

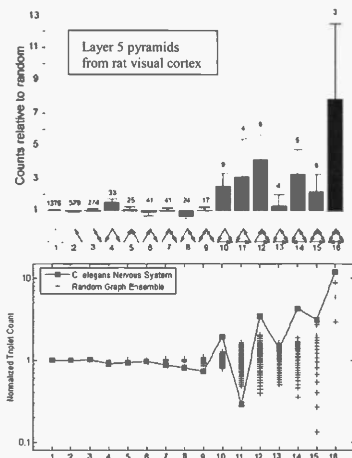

FIGURE 3 We discovered similar overrepresented connectivity patterns, or motifs, in rat neocortex and *C. elegans*. These motifs may reflect general computational constraints on the circuits.

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BRAIN DEVELOPMENT AND PLASTICITY

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My lab is interested in determining the function of experience in regulating brain development. It is now commonly understood that sensory experience shapes the development of circuits within the brain and that experience during brain development governs brain connectivity, perception, cognition, and behavior. Nevertheless, the experience-dependent cellular and molecular mechanisms that operate to establish and modify brain connections during development are poorly understood. By understanding the basic mechanisms governing brain development, we expect to garner critical information of the origins of nervous system dysfunction, as seen in many neurological disorders.

We examine the structural and functional development of the visual system in amphibian tadpoles. These animals are transparent, which allows us to observe directly the development of the brain in living animals. In addition, we assess neuronal function using electrophysiological assays of synaptic connectivity and synaptic plasticity. We combine these studies with gene transfer methods 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. Below is a representative project completed in the laboratory during the last year.

STRUCTURAL DEVELOPMENT OF MOTOR NEURON AXONS USING TIME-LAPSE IN VIVO IMAGING

The development of motor neuron axon arbors governs the connectivity between the central nervous system and the musculature. Once the motor neuron axon reaches the target, the growing axon branches and forms synapses. In many cases, it is thought that exuberant connections are made and that these are pruned to shape the final axon arbor and neuromuscular connections. The development of the motor neuron axon arbors has been characterized as a sequence of events in which the axons initially branch exuberantly, followed by a period of synapse and branch elimination; however, the initial phase of axon arbor elaboration and synaptogenesis has not been directly observed in vivo. 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, has not been addressed. To test this hypothesis, we collected time-lapse images of motor neuron axons as they elaborated complex arbors and formed neuromuscular synapses.

We found that motor neuron axon terminal arbors show continuous branch dynamics over 3 days of time-lapse imaging. Using a combination of cyan-fluorescent protein-tagged synaptophysin (synaptophysin-CFP) and yellow fluorescent protein (YFP) to visualize the dynamics of presynaptic structures and axon branches and Texas-Red-tagged α-bungarotoxin (TR-αBTX) to label postsynaptic acetylcholine receptors (AChR), we show that new branches emerge from sites of presynaptic specialization, the majority of which are apposed to AChR. The new branches form neuromuscular synapses. These data suggest that mechanisms that govern neuromuscular synaptogenesis also have an important role in regulating motor neuron axon arbor development.

To probe the role of synaptogenesis in controlling axon arbor development, we tested whether a protein called candidate plasticity gene 15 (CPG15, also known as Neuritin) affects this process. We previously demonstrated that CPG15 enhances the development of visual system connections by increasing the elaboration of optic tectal cell dendrites, retinal axon arbors, and the strength of retinotectal synapses. CPG15 is expressed in the developing motor neurons. CPG15 expression enhances the development of motor neuron axon arbors by promoting neuromuscular synaptogenesis and by increasing the addition of new axon branches. These data indicate that motor neuron axon elaboration and synaptogenesis are concurrent and iterative.

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Shu-Ling Chiu, WSBS student, Holly Cline's lab.

GENETICS OF MEMORY IN *DROSOPHILA*

J. Dubnau A. Altick
A. Blum
S. Dennison

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

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 a growing number 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. Ultimate confirmation that a gene is involved in memory formation rests with the demonstration that in vivo modulation (disruption) of the gene alters that process with some specificity. With that aim in mind, we are focusing on local translational control, one of several pathways suggested from the array experiments. Our genetic studies already support a role in memory for several components of this pathway. These include *staufen* and *oskar*, 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.

Drosphila 4E-BP

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

thor, which encodes a fly homolog of 4E-BP, a translational regulator, is one of the CMGs induced by spaced training relative to massed training. 4E-BPs bind to 4E, the cap-binding protein, thereby inhibiting translation. Upon phosphorylation, however, 4E-BPs are removed from the complex, allowing 4G to interact with 4E, and translation initiation to occur. We have demonstrated that a hypomorphic mutation in *thor* yields a defect in long-term memory measured 24 hours after spaced training (Fig. 1). Importantly, learning and shorter-term forms of memory, which are believed to be protein-synthesis-independent, are normal in these mutant animals. We now are using a dominant-negative transgenic form of *thor* to block 4E-BP-mediated translation. This transgene, which con-

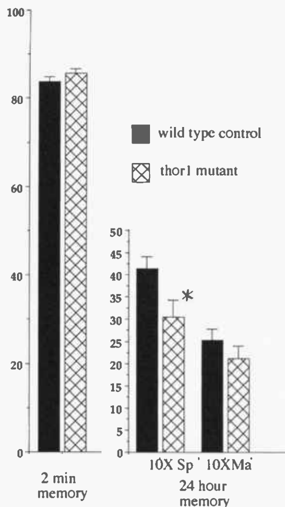

FIGURE 1 *thor* is a long-term memory mutant. *thor1* mutant animals display normal levels of memory measured 2 minutes after a single training session. In contrast, *thor1* mutants exhibit defective long-term memory. Memory is reduced in *thor1* mutants when measured 24 hours after ten sessions of spaced training (10XSp), but not after ten sessions of massed training (10XMa).

tains an amino acid substitution at the site of phosphorylation, blocks 4E-BP-mediated translation by constitutively binding 4E. This transgenic approach will allow us to acutely inactivate 4E-BP-mediated translation during memory formation and also will provide the means to spatially restrict expression to distinct neuroanatomical loci.

Genetic Dissection of Anesthesia-resistant Memory

A. Blum, J. Dubnau

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 1 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. Until now, nothing was known about the molecular, genetic, or cell biological pathways underlying ARM. We have identified *radish* as a phospholipase-A2, providing the first clue about signaling pathways underlying ARM in any animal (Chiang et al. 2004). An enhancer-trap allele of *radish* (C133) also provides an entry point to study the anatomical circuits underlying ARM. rsh^{C133}-driven reporter expression does not label mushroom bodies, which are the primary anatomical focus of olfactory memory research in *Drosophila*. Instead, rsh^{C133}-driven expression reveals a number of novel neuronal types, several of which project into mushroom bodies. Transgenic expression of PLA2 driven by the rsh^{C133} Gal4 driver restores normal levels of ARM to *radish* mutants, whereas transient disruption of neural activity in rsh^{C133} neurons inhibits memory retention. Identification of *radish* as a phospholipase-A2 and the neural expression pattern of this enhancer trap significantly broaden our understanding of the biochemistry and anatomy underlying olfactory memory in *Drosophila*. These findings also are consistent with pharmacological studies of PLA2 signaling in memory formation in vertebrate animals. Our current efforts are focused on a biochemical characterization of the *radish* PLA2, as well as an anatomical dissection of the neural circuits in which *radish* functions.

Reversible Disruption of Synaptic Transmission

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

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 a time scale of minutes. We are using a panel of Gal4 enhancer drivers to focus expression of this dynamin transgene within specific subpopulations of neurons. With this approach, we are now able to reversibly silence reproducibly specific groups of neurons in vivo. We already

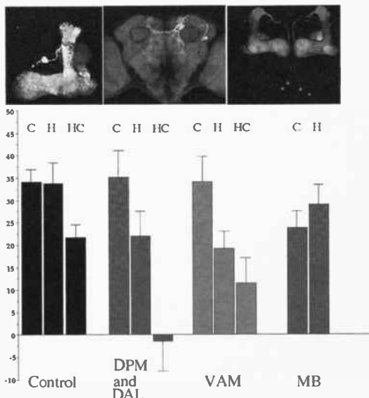

FIGURE 2 Anatomical dissection of anesthesia-sensitive and anesthesia-resistant memory (ARM). (*Top panels*) GFP reporter expression in several neurons that participate in the circuitry underlying olfactory memory. (*Left panel*) Dorsal-paired medial neuron massively innervates the axonal outputs of the mushroom bodies. (*Middle panel*) Dorsal-anterior-lateral neuron sends fibers into parts of the posterior superior protocerebrum, including the mushroom body dendritic field. (*Right panel*) Ventral-anterior-medial neurons innervate the alpha lobes of the mushroom bodies (gray). (*Bottom panel*) Expression of the temperature-sensitive dynamin transgene under control of Gal4 drivers permits focused transient inhibition of dynamin-dependent activity in specific neuronal subtypes. Three Gal4 lines were used: one that expresses in DPM and DAL, one that expresses in VAM, and a third that expresses in mushroom bodies. In the case of the DPM and DAL line as well as the VAM line, transient heating to the restrictive temperature (H) causes a partial amnesia measured 3 hours after training (compare control treatment [C] with heat shifted [H]). In contrast, this heat shift has no effect on a control strain or on a line that expresses the dynamin transgene in mushroom bodies. Application of a cold-shock anesthesia cold-shock treatment completely erases anesthesia-sensitive memory, providing a pure measure of ARM. Use of cold shock in combination with the heat shift (HC) reveals an anatomical dissection of anesthesia-sensitive memory from anesthesia-resistant memory. In the DPM/DAL expressing animals, this combination treatment erases all memory, suggesting that the memory disrupted by dynamin alone (H) is ARM. In contrast, the combined treatment in VAM neurons reveals that a significant portion of the memory remaining after the dynamin effect is ARM. Thus, inhibition of VAM-dynamin mostly disrupts anesthesia-sensitive memory.

have demonstrated a role for synaptic transmission in mushroom 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 long-term memory phases—and nothing is known about the circuitry of memory retrieval.

In collaboration with A.S. Chiang, we have identified a number of Gal4 enhancer lines that yield 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 now can functionally manipulate these neuronal populations as well as a number of additional neuroanatomical loci identified by reporter expression from a panel of approximately 60 memory mutants (Dubnau et al., *Curr. Biol. 54:* 238 [2003]). Using this method of reversible neuronal disruption, we already have demonstrated an anatomical dissection of the requirements for dynamin-dependent activity during formation and storage of anesthesia-sensitive versus anesthesia-resistant memory (Fig. 2). 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.

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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. This 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 use flies, frogs, and mice as models to study signals that regulate distinct steps in the differentiation cascade and mediate the interactions between the stem cells and their microenvironment. Our main focus is on stem cells in the adult brain. We have generated several models to study how stem cells decide to give rise to progenitors and, ultimately, neurons. We also use these models to study how disease and therapeutic drugs affect adult neurogenesis.

Much of our interest is related to a versatile signaling molecule, nitric oxide (NO). We found that, in addition to its numerous other tasks such as fighting infection, regulating blood pressure, and mediating neurotransmission, NO controls cell proliferation and differentiation. In several developmental settings, NO suppresses cell division, helping to control the balance between proliferation and differentiation. For instance, we have found that NO acts as an negative regulator of cell division in the adult mammalian brain, such that by experimentally reducing NO levels, we can increase the number of neural stem/progenitor cells in the adult brain. Our findings highlight the role of NO as an essential negative regulator of cell proliferation in the differentiation cascade. We are now beginning to dissect the genetic and molecular interactions between NO and the signaling pathways that control stem cell division and differentiation.

STEM CELLS IN THE ADULT BRAIN

Stem and progenitor cells receive specific cues that control their self renewal and their transition through differentiation cascades. We are interested in stem cells in the adult organism and the signaling molecules that control the division of stem and progenitor cells and guide them toward their differentiated fate. To follow neuronal differentiation, we generated a series of transgenic reporter mouse lines in which neural stem and early progenitor cells of the embryonic and adult brain are marked by the expression of the green, cyan, and red fluorescent proteins (GFP, CFP, RFP) which are under control of the nestin gene regulatory elements. We found that these labeled cells faithfully report the status of neural stem cells after exposure to drugs or in response to gene ablation.

We also found that in some nonneural tissues of our model animals, fluorescence marks stem/progenitor cells for these tissues. For instance, we found that nestin-GFP-expressing cells can be found in the bulge region of the hair follicle, where stem cells for the follicle are located. By a number of criteria, these cells indeed behave as progenitor population of the hair; in addition, they can generate progeny of diverse lineage, including neurons. We used transcriptional profiling to confirm the neural potential of these cells and to determine their relation to other types of stem cells. We now study the ability of nestin-expressing cells from nonneural tissues to reveal their neural potential both in vitro and in vivo, after transplantation into the developing and adult mouse brain.

An important focus of our studies remains the role of NO as a regulator of adult neurogenesis. We have recently found that NO acts to suppress the generation of new neurons in the adult brain. In a pharmacological approach, we blocked production of NO by introducing inhibitors of NO synthase (NOS) into the ventricles of the adult brain. In a genetic approach, we generated a null mutant for the neuronal NOS gene. In both models, the number of new cells generated in neurogenic areas, the olfactory subependyma and the dentate gyrus, was strongly augmented. Importantly, increased proliferation of progenitor cells did not alter the fate of the extra cells. Our results reveal a role for NO as an essential negative regulator controlling production of new neurons in the adult brain. This finding parallels our recent results indicating that NO also acts as a negative regulator of stem/progenitor cells in the bone marrow. It also suggests a strategy for enhancing

neurogenesis in the damaged central nervous system. At the same time, it is unclear which steps within the neural differentiation cascade are controlled by NO: It may control the last steps in the cascade (for instance, permitting advanced precursors cells to proceed with an extra division) or it may act upon stem or very early progenitor cells, accelerating their rate of division. Furthermore, it is not known whether NO is produced in the differentiating cells themselves, or in neighboring cells (thus acting on stem or differentiating cells in a transcellular fashion). We now use our reporter lines (described above) to determine whether deficit of NOS activity changes the number of neural stem/progenitor cells in the adult brain or affects more advanced neuronal precursors. Our results point to a specific step in the differentiation cascade that is controlled by NO. We are now applying a similar strategy to investigate how therapeutic drugs and selected genes affect adult neurogenesis and to determine their points of action within the neuronal differentiation cascade.

NO AND EARLY *XENOPUS* DEVELOPMENT

Vertebrate body plan is shaped during embryogenesis through precise coordination of cell proliferation and morphogenetic cell movements. We investigated the role of NO in early *Xenopus* development using gain- and loss-of-function approaches by microinjecting either mRNA encoding *Xenopus* NOS (XNOS) or a chemical NO donor; conversely, we blocked NOS activity by injecting either mRNA encoding a dominant-negative form of XNOS, or chemical NOS inhibitors. Our results show that NO suppresses cell division and regulates cell movements during early development of *Xenopus*. Excess NO production decreases cell proliferation in the embryo. In contrast, deficits in NO production result in excessive cell proliferation in the ectoderm and distortion of cell movements that underlie normal axis extension and neural tube closure during gastrulation and neurulation. At later stages, the deficit of NO leads to profound defects in organogenesis. We have identified molecular targets and pathways used by NO to control cell cycle and cell motility. Our results indicate that NO-RhoA-ROCK signaling mediates both convergent extension and cell division. Concurrent control by NO helps ensure that the crucial processes of cell proliferation and morphogenetic movements are coordinated during early development.

NO AND *DROSOPHILA* DEVELOPMENT

NO is an essential regulator of *Drosophila* development and physiology: It has been implicated in visual system development, immunity, behavior, response to hypoxia, osmoregulation, and regulation of cell cycle progression during development. We found a novel mode of regulation of NOS function that employs endogenously produced truncated protein isoforms of *Drosophila* NOS (DNOS). These isoforms inhibit NOS enzymatic activity in vitro and in vivo, reflecting their ability to form complexes with the full-length DNOS1 protein. Truncated isoforms suppress the antiproliferative action of DNOS1 in the eye imaginal disc, yielding hyperproliferative phenotypes in pupae and adult flies. We found that NO regulates the cell cycle by acting upon the retinoblastoma pathways, suppressing the effect of RBF and enhancing the effect of E2F. Our results indicate that endogenous products of the DNOS locus act as dominant-negative regulators of NOS activity during *Drosophila* development, pointing to a novel mechanism for the regulation of NO production in animal cells. More generally, negative control of homodimeric enzymes by endogenous truncated isoforms may represent an important regulatory mechanism controlling development and physiology.

In a related collaborative study with Michael Regulski and Tim Tully here at CSHL, a wide screen for lethal EMS-induced mutations around the DNOS locus produced a set of lethal mutations in five complementation groups, the largest group corresponding to DNOS. Sequence analysis of the genomic DNA of one of these lines identified a single nucleotide transition which created a Gly1942Glu substitution. This residue is conserved across all known NOS proteins; although it does not fall into the active center of enzyme, its mutation abolishes the enzymatic activity of DNOS. These results provide the first direct evidence that NOS function is indispensable for development.

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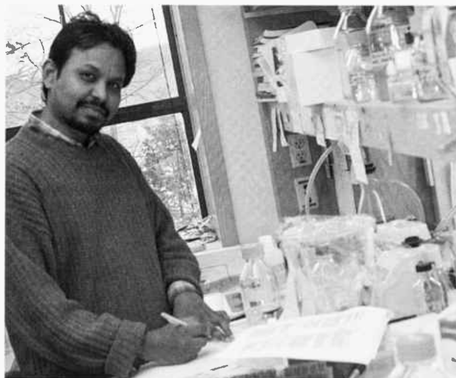

Kandasamy Ravi, postdoc, Grisha Enikolopov's lab.

CONSTRUCTION AND PLASTICITY OF THE GABAERGIC CIRCUITS

Z.J. Huang J.-R. Ango, B. Chattopadhyaya, G. diCristo, S. Kulhman, C. Wu, P. Wu, H. Yang

Much progress in neuroscience in the past few decades has been made in understanding information coding, storage, and plasticity in the brain. Glutamatergic synapses on principal neurons—with their vast numbers, elaborate electrobiochemical signaling machinery, and capacity for modification— have been a major focus of investigation. On the other hand, perceptual and cognitive processes also require large neural networks to dynamically retrieve and combine various stored information, and to relate them to incoming sensory input, usually with high speed and temporal precision, on the order of tens of milliseconds. Evidence in the past few years increasingly suggests that the GABAergic system is particularly effective in regulating the precise timing of electrical signaling both within a principal neuron and among populations of principal neurons. Networks of GABAergic interneurons have been hypothesized to entrain populations of principal neurons and thus impose a coordinated "context" for the "content" carried by networks of principal neurons during perception, cognition, movement control, learning, and memory. Aberrant GABAergic functions have been implicated in devastating neurological and psychiatric disorders such as epilepsy and schizophrenia.

Our laboratory is studying the assembly, plasticity, and function of the GABAergic inhibitory network in mammalian brain. We focus on the following three areas: (1) mechanisms of subcellular organization of distinct classes of GABAergic synapses; (2) mechanisms of neuronal activity-dependent plasticity of GABAergic synapses; and (3) role of GABAergic function in critical-period plasticity in primary visual cortex. Combining cell-type-specific transcriptional promoters, bacterial artificial chromosome (BAC) transgenic reporter mice, and high-resolution imaging, our laboratory has established powerful in vivo and in vitro systems to visualize and manipulate defined classes of GABAergic interneurons. Our progress in the past year is beginning to define the logic and outline of the construction of GABAergic circuits in the mammalian brain.

Subcellular Synapse Organization Is Independent of Experience-driven Pruning

G. diCristo, C. Wu

The subcellular spatial organization of GABAergic innervation is crucial for the temporal precision in their regulation of neuronal excitability, integration, and synchrony. Since subcellular synapse targeting is preceded by a largely genetically determined axon guidance process and followed by synapse formation and experience-driven plasticity, it is not obvious whether this intermediate step of circuit assembly is directed mainly by genetic programs or experience-dependent refinement. By combining cell-type-specific promoters, BAC engineering, and 2-photon imaging, distinct classes of GABAergic synapses and their postsynaptic pyramidal neurons were visualized in primary visual cortex and in organotypic cultures of transgenic mice. We show that the subcellular organization of perisomatic and dendritic-targeted GABAergic innervations in organotypic cultures was indistinguishable compared to that in primary visual cortex. Therefore, subcellular targeting of GABAergic synapses is likely guided by cell surface molecular labels and experience-independent forms of neuronal activity.

Subcellular Organization of GABAergic Synapses Directed by L1CAMs and Ankyrin-based Membrane Skeletons

J.-R. Ango, H. Yang, P. Wu

We are defining the molecular signals underlying subcellular synapse targeting. We have discovered so far that the ankyrinG-based membrane cytoskeleton is the key molecular machinery which, through subcellular recruitment of the L1 family immunoglobulin cell

adhesion molecules (IgCAMs), directs GABAergic innervation to the axon initial segment—the site of action potential initiation. Importantly, both ankyrin and L1CAM families contain multiple members localized to distinct subcellular compartments. We have significant evidence that CHL1 (a close homolog of L1) is involved in targeting a different class of GABAergic synapses to neuronal dendrites. Together, these results led us to propose a general hypothesis: Members of L1CAMs recruited to subcellular domains by different ankyrins may constitute a set of "compartmental code" in principal neurons to direct subcellular organization of GABAergic synapses.

An important implication of these discoveries is that subcellular segregation of GABAergic synapses is superimposed on an elaborate subcellular organization of ion channels. Such spatial alignment of biophysical properties and synaptic inputs, in some cases upon the same macromolecular complex, may confer the ultimate temporal precision of neuronal signaling within and among neurons. These studies suggest an unexpected link from subcellular molecular organization, neuronal connectivity, to network properties. In addition, there should be a family of receptors on different classes of GABAergic neurons to "read" the compartmental code.

Neuronal Activity-dependent Plasticity of GABAergic Synapses and Circuits

B. Chattopadhyaya, G. diCristo, C. Wu

Although precision and speed of transmission are prominent features of at least certain classes of GABAergic neurons, it has been well documented that experience and injury profoundly alter the connectivity and function of GABAergic synapses. The logic and mechanism of neuronal activity-dependent plasticity of the GABAergic network are poorly understood.

Using high-resolution imaging of a defined class of GABA synapses, we discovered that following the initial targeting to a defined subcellular domain, there is a prolonged maturation of GABA innervation, characterized by elaboration of multiple terminals and proliferation of synapses. This maturation process can proceed to a substantial extent in an organotypic culture system, but it is regulated by level/pattern of neuronal activity and sensory experience during a restricted postnatal period in visual cortex. Using "single-cell genetics" in organotypic cultures, we are making significant progress in understanding the molecular mechanism of activity-dependent morphologic plasticity of a major class of GABA synapses. First, we discovered that, although GABA transmission is "inhibitory," GABA synthesis and signaling are essential in synaptic innervation and maturation (Fig. 1). This unexpected finding suggests that in addition to its role as an inhibitory neurotransmitter, GABA has a key role in axon branching, synapse proliferation, target coverage, and thus the construction of circuits. Second, we discovered that the Fyn tyrosine kinase, neural cell adhesion molecule (NCAM), and its polysialic acid (PSA) modification all have a role in the morphologic plasticity of GABAergic presynaptic terminals. These results begin to suggest a pathway in which activity-dependent regulation of GABA synthesis and signaling through the GABAB receptors and G proteins could regulate Fyn kinase activity, which then modulates the voltage-gated calcium channel and therefore Ca^{2+} dynamics in presynaptic terminals. The spatial and temporal dynamics of Ca^{2+} signaling have been shown to trigger a variety of morphogenic events.

Our studies suggest that the logic and mechanism of the plasticity of GABAergic synapses may be fundamentally different from that of the glutamatergic excitatory synapses. For example, synapse specificity in the plasticity of glutamatergic synapses is the basis for information coding and storage. However, the plasticity of the GABAergic synapses that we observed is not restricted to particular terminals, but "cell wide" and, in this sense, global. These results have important implications in the function of GABAergic plasticity on neural network activities.

GABAergic Transmission and Critical-period Plasticity in Visual Cortex

S. Kuhlman

During early postnatal life, visual deprivation alters synaptic transmission in visual cortex such that binocular cortical neurons cease to respond to stimulation of the deprived eye. Long-term depression (LTD)

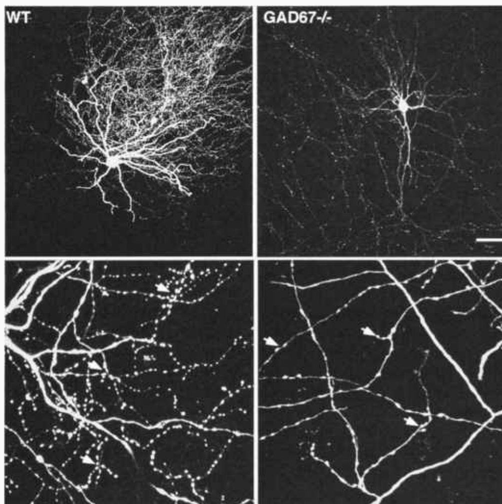

FIGURE 1 GABA synthesis and signaling influence the construction of GABAergic circuits. (*Left*) A wild-type basket interneuon projects highly exuberant local axon arbors and branches, which bear dense synaptic boutons (*arrow*). Deletion of a GABA synthetic enzyme in a single basket cell (*right*) greatly reduces its axon terminal branches and synaptic boutons, and thus the innervation of its target neurons.

induced via spike-timing-dependent plasticity (STDP) rules has been implicated in competition-based Hebbian plasticity and selective weakening of deprived input. However, the precise mechanisms by which input from the two eyes compete within the cortical circuit for control of postsynaptic action potential is not known. GABAergic transmission has been implicated in the temporal precision of spike generation and is necessary for ocular dominance (OD) plasticity, but the cellular mechanism is obscure.

We hypothesize that rapid and transient recruitment of GABAergic inhibition by open eye input suppresses the deprived pathway from driving postsynaptic spikes, resulting in synaptic depression via STDP rules. We developed a two-pathway stimulation paradigm at layer 4 to layer 2/3 connections in visual cortical slices and demonstrated that there is a developmental shift in the interaction of converging synaptic inputs, from summation/coordination to suppression/competition. This developmental increase of competition is in part mediated by the maturation of GABAergic inhibition, which dynamically regulates spike generation. Modeling studies further suggest that mature GABA transmission promotes depression of uncorrelated synaptic inputs. We propose that GABAergic inhibition recruited by correlated input is a potent competitive mechanism to suppress spike probability of uncorrelated input, thereby engaging molecular cascades leading to synaptic depression. This study reveals a plausible GABAergic mechanism in critical-period plasticity, which is distinguishable from (but regulates) the well-known "core plasticity machinery" at glutamatergic synapses and neurons.

Genetic Logic of GABAergic Cell Types and Gene Expression Profiles

J.-R. Ango, P. Wu [in collaboration with Dr. S. Nelson, Brandeis University]

We continue to generate BAC transgenic mice which label different classes of interneurons with fluorescent proteins. We have initiated a new effort to characterize gene expression profiles in several classes of GABAergic interneurons. This was made possible by developing a procedure to manually purify GFP-labeled interneurons from our BAC transgenic reporter mice. A systematic understanding of gene expression profiles among different classes of interneurons throughout development and in response to stimulation will yield fundamental insight into the logic of the design and function of the GABAergic system.

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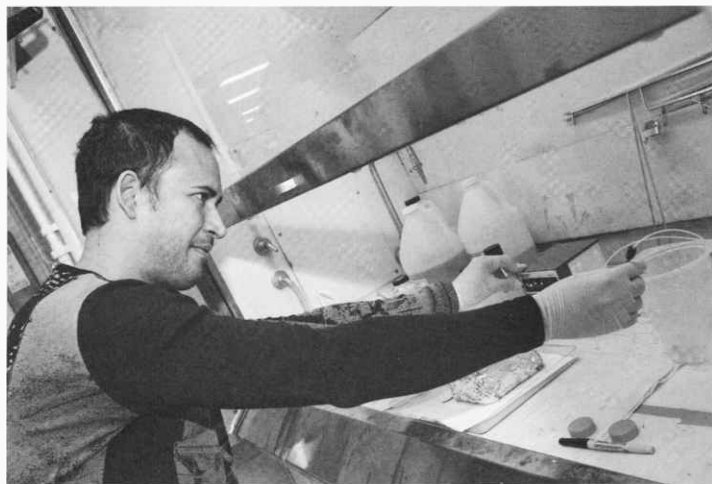

Jean-Raymond Ango, postdoc, Josh Huang's lab.

BIOPHYSICAL BASIS FOR NEURAL COMPUTATION

A. Koulakov M. Nikitchenko
D. Tsigankov

Neurons in the brain display the sort of duality that has inspired philosophers for many centuries. On the one hand, neural networks process information. In doing so, they represent our ideas, feelings, and thoughts. On the other hand, information is processed by a finely tuned physiological apparatus, extremely sophisticated per se. It is not even clear what level of function of the nervous system is more complex, information processing or physiological organization. In our lab, we attempt to connect these two features of the nervous system. We argue that the latter often provides cues about the former and vice versa, and that these two components cannot be understood without one another.

Hysteretic Model for Neural Integrator

M. Nikitchenko, A. Koulakov

Persistent firing is a likely candidate for the neural correlate of short-term memory. In some cases, the variable stored in memory is continuous in nature. Examples of such variables include position of an object in the sensory space and tension of a muscle, or variables representing accumulated sensory evidence. Such variables are encoded in persistent neuronal firing, which has a graded set of values. Perhaps the best-studied system of this type is the oculomotor neural integrator. Graded persistent activity in this system is likely to be maintained by positive feedback. This poses a basic problem of robustness, since mistuning of the feedback leads to instabilities, which are hard to avoid in realistic systems. It was hypothesized that the problem of robustness could be solved by introducing hysteresis into neuronal responses. A recent study in the goldfish oculomotor integrator (Aksay et al., *Cerebr. Cortex 13:* 1173–1184 [2003]) may determine whether hysteretic mechanism of robustness is indeed used. Two basic observations are made about history dependence of neuronal responses in this system: (1) firing rate of one neuron versus the other exhibits hysteresis and (2) the size of this hysteresis varies from a pair of cells to a pair of cells. In this study, we develop a mathematical approach, which allows us to address these observations. We derive a kinetic equation, which can describe an ensemble of hysteretic units effectively and accurately. Using this approach, we consider dynamics of an ensemble of hysteretic units with different values of hysteresis, similarly to the experiments in goldfish. We conclude that such an ensemble exhibits history dependence of firing rates, including hysteresis in the firing rate of one cell versus the other. Thus, experimental observation number one, cited above, may follow from observation number two. This conclusion follows from entanglement of the distribution function of activated units in the phase space of the system. We confirm this conclusion by studying an integrator based on a single neuron with 100 hysteretic dendritic compartments.

Complete Structure of Topographic Maps in Ephrin-A-deficient Mice

D. Tsigankov, A. Koulakov

Axons of retinal ganglion cells establish orderly projections to the superior colliculus of the midbrain. Axons of neighboring cells terminate proximally in the superior colliculus, thus forming a topographically precise representation of the visual world. Coordinate axes are encoded in retina and in the target through graded expression of chemical labels. Mapping based on chemical labels alone does not yield required specificity of connections. Additional sharpening is provided by electrical activity, which is correlated between neighboring axons. In this work, we propose a quantitative model that allows combining the effects of chemical labels and correlated activity in a single approach. Using this model, we study a complete structure of two-dimensional topographic maps in mutant mice, in which one of the labels, encoding a horizontal retinal coordinate (ephrin-A), is reduced/eliminated. We show that topographic maps in ephrin-A-deficient mice display granular structure, with the regions of smooth mapping separated by linear discontinuities reminiscent of fractures observed

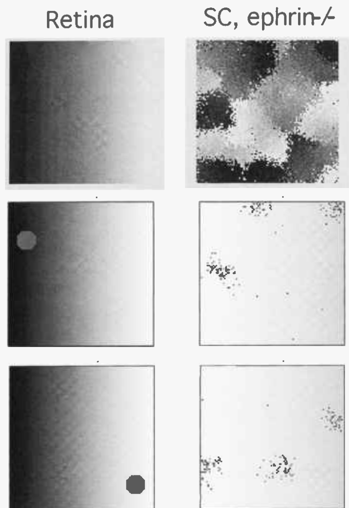

FIGURE 1 Topographic maps in ephrin-A2/A5 –/– knockout mice are analyzed by a computational model that includes chemicals labels and correlated activity-based refinement. The resulting map contains domains of continuous mapping separated by linear discontinuities similar to fractures in orientation preference maps in other mammals, with more complex organization of visual maps. Thus, a loss-of-function experiment becomes a gain-of-function experiment. SC indicates superior colliculus.

in the maps of preferred orientation. We also demonstrate the presence of point singularities, similar to pinwheels in orientation maps.

Activity and Competition in Surgically Manipulated Retinotectal/Collicular Maps

D. Tsigankov, A. Koulakov

We analyze a theoretical model of retinotectal/collicular map development in surgically manipulated systems. Our model is based on the total affinity of retinal ganglion cell axons to their targets in tectum. This total affinity includes chemoaffinity, activity-dependent Hebbian contribution, and competition for positive factors in the target. We show that many observed phenomena may be described in the same framework in terms of the interplay of different contributions to the total axonal affinity. Thus, after removal of a part of the retina or tectum, the final topographic map is stretched or compressed. This behavior results in our model from the axonal competition for positive factors, which is similar to one considered for the neuromuscular junctions, and chemoaffinity. The competition maximizes the number of branches for each axon, ensuring approximately the same number of them for every axon. Chemoaffinity arranges these connections in a topographically precise order. In another experiment, when lesions are made in the contralateral retina, the sprouting capacity of the ipsilateral axons is increased after chronic NMDA (*N*-methyl-D-aspartate) receptor blockade. In our description, this phenomenon appears to be due to interplay between the axonal competition and activity-dependent Hebbian contribution to the total affinity. The activity block eliminates the competitive advantage of the contralateral axons and leads to the increased sprouting capacity of the ipsilateral axons. Finally, if the part of the tectum is surgically reimplanted after a 180° rotation, as was done in goldfish, the activity-independent and -dependent mechanisms may themselves oppose one another. The resulting map within the reimplanted region is reversed if the region size is greater than some critical value, and it is unchanged otherwise. Thus, we propose the description that unites the activity-independent and -dependent mechanisms of the retinotectal/collicular map formation together with axonal competition within the same framework.

Automatic Alignment of Serial-section Electron Micrographs

A. Koulakov [in collaboration with D. Chklovskii, Cold Spring Harbor Laboratory]

Electron microscopy (EM) of ultrathin serial sections produces a series of misaligned micrographs. This happens because sections are placed on the grid somewhat irregularly and because the sections are subject to physical distortions. Therefore, the three-dimensional reconstruction requires section alignment, or registration. We designed an algorithm for fully automatic registration of stacks of serial-section EM images. Our algorithm is based on sequential pairwise alignment of neighboring images in the stack. One of the images in the pair remains unchanged during the

alignment procedure (reference), whereas the other is transformed using linear (shift, rotation, magnification, and shear) and polynomial (2nd and 3rd degree) transformations. The quality of alignment is assessed using a measure of similarity between the reference and transformed images. As a measure of similarity, we use normalized cross-correlation. To perform alignment automatically, we maximize cross-correlation with respect to the parameters of transformations. To implement the search of the optimal set of parameters, we use two popular optimization methods: simulated annealing and genetic algorithm. In this work, we compare the performance of the annealing and genetic procedures on the stack of EM images and compare various types of spatial transformations: linear, 2nd, and 3rd degree polynomial. The same method with small modifications can be used to repair images containing substantial tears and folds. We describe an algorithm and show results of fully automatic recovery of such images.

Analysis of Mitral Cell Odor Responses in Behaving Mice: Spikes and Oscillations

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

We performed extracellular recordings in the olfactory bulb of awake behaving mice. The water-deprived mice were trained in a computer-controlled olfactometer to discriminate odorants. A microdrive with three electrodes that could be moved independently by three micromotors was implanted to record from the olfactory bulb. Responses of cells from the mitral cell layer were recorded from three electrodes simultaneously together with behavioral events.

We addressed the question of how neuronal responses recorded in behaving mice contribute to odor discrimination. We distinguished three components of the responses recorded simultaneously on all electrodes: breathing pattern at approximately 3–4 Hz, oscillations of the local field potential (LFP) at 50–60 Hz, and neuronal action potentials. We observed the following: (1) Firing rate and LFP oscillations are modulated by behavioral events and correlated with the stimulus. This information can be used to discriminate the odorants. (2) The amplitude of LFP oscillations is modulated by the breathing pattern. (3) The timing of action potentials is correlated with the phase of LFP oscillations. (4) Oscillations on different electrodes are correlated but not identical. These observations provide insights into invariants and variability in neural representations of olfactory stimuli.

Mitral Cell Responses in Awake and Anesthetized Mice

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

The vast majority of our knowledge about the function of neurons in the mammalian olfactory bulb was obtained in anesthetized preparations. A few recordings from mitral cells in behaving animals suggest that neuronal dynamics is quite different in behaving animals compared to anesthetized animals.

A microdrive with three electrodes that can be moved independently by three micromotors was implanted to record from the mouse olfactory bulb. The water-deprived mouse was previously trained in a computer-controlled olfactometer to discriminate multiple odors. The implanted microdrive allows us to record activity of individual cells for 1–5 days. After a behavioral session, a mouse is anesthetized, and the responses of the same cells that were recorded in the awake animal are recorded in the anesthetized animal.

Recording of mitral cell activity in anesthetized and awake states showed a striking difference. First, the spontaneous activity in the awake state was much higher and did not show an obvious synchronization with the breathing. Second, the odor tuning of the mitral cells was much broader in the anesthetized state. A cell responsive to an odor onset in the anesthetized state did not respond to the same odor in the awake state. Third, the same cells changed their activity in the awake state in response to behavioral cues.

A simple phenomenological model is proposed to explain our results, and implications for the olfactory code in the awake animal are discussed.

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THE NEURAL ORGANIZATION OF OLFACTORY BEHAVIOR

Z.F. Mainen V. Egger S. Ranade
C. Feierstein D. Sosulski
H. Gurden N. Uchida
A. Kepecs H. Zariwala
M. Quirk

Our laboratory is interested in the neural basis of mammalian behavior. We focus on understanding the nature of the electrical activity of single neurons, the chief currency through which genes and molecules express themselves in the functioning brain. We are using the rodent olfactory system as a model for studying neural coding, the problem of how information is represented in neuronal spike trains. We view neural coding in the broader framework of decision making, that is, how neurons work together to select one of a repertoire of possible motor actions on the basis of experience, current goals, and sensory data. We are especially interested in the temporal coordination of activity between neurons and between regions of the brain that are necessary for coherent and directed action. Our principal experimental technique is chronic multi-electrode recordings from rodents trained to perform sensory discrimination tasks. We are actively developing new ways of monitoring and controlling both behavior and neural activity. We are also using intrinsic optical imaging to study the link between electrical activity (information) and metabolic activity (energy) in the brain and two-photon imaging and whole-cell recording to study the synaptic and electrical properties of individual neurons. To help understand the relationship between neural and behavioral phenomena, we are using neural network and statistical theories that can be expressed in computational models and are thereby related to our data. Our long-term goal is to apply these approaches and insights to human psychiatric disease, especially schizophrenia.

Psychophysical Constraints on Olfactory Coding

N. Uchida

How is odor information coded in the brain? Psychophysical experiments can have a crucial role in elucidating neural coding because behavior is the ultimate readout of the neural code. Reaction time experiments, in which the time it takes to make a decision is measured, can provide particularly crucial constraints on the temporal aspects of neural coding. Olfaction is sometimes thought of as a slow sense, and thus it has been proposed that relatively slow temporal processing might play a role in odor coding. We recently tested this idea using an odor mixture discrimination task in rats (Fig. 1). We found that across a wide range of conditions, rats discriminated odors very rapidly, with a median odor sampling duration of <300 ms (Uchida and Mainen *Nat. Neurosci. 6:* 1224 [2003]). Measurement of the rats' sniffing activity revealed that optimal performance was achieved with a single sniff at theta frequency (8–10 Hz). Since this original study, we have continued to systematically explore parameters of the task that might impact the speed and accuracy of olfactory decisions. These studies have led us to focus on the contribution of motivation in determining choice behavior (see below).

In a related set of experiments, we tested the rat's behavioral strategy in an odor mixture discrimination task. After training, rats were tested using probe stimuli that possessed different absolute odor component concentrations but identical odor component ratios as the stimuli used in the training set. Performance was predicted by a ratio-based discrimination strategy but not by alternatives such as an intensity-based discrimination strategy. This result indicates that molar ratios of the components of a mixture are the critical determinants of choice in this task. We propose that odor quality is coded by the ratio of receptors that bind the different odorants present in a stimulus, that is, a "ratio coding" scheme based on how many receptors bind to molecules of odor A versus how many bind molecules of odor B when a mixture containing odors A and B is presented. To understand how ratio coding might arise from ensemble activity in the olfactory bulb and cortex, we are currently recording spike activity in behaving rats using multi-electrode techniques.

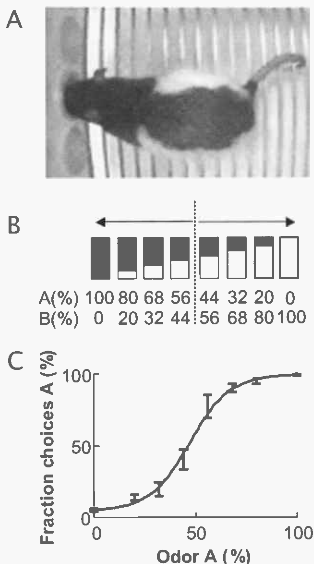

FIGURE 1 Two-alternative odor mixture discrimination task. (*A*) A rat performing odor discrimination task. Rats can perform this task with 200 ms of odor sampling regardless of discrimination difficulties. (*B*) Mixture stimuli. Two odors were mixed with various proportions and rats were trained to report the dominant component by making left or right a choice. (*C*) Typical performance in odor mixture discrimination tasks.

Effects of Sensory and Reward Experience on Olfactory Decisions

H. Zariwala, N. Uchida, A. Kepecs

Although one might think of the job of the rat in our task as determining the identity of the odors it is presented, behavioral ecology suggests that the rat is trying to obtain as much water as fast as it can. That is, animals make foraging decisions to maximize reward and minimize work. Therefore, to understand the decisions that rats make in this task, one must consider the sensory information in the context of the incentives afforded by the task. We have begun to systematically investigate how olfactory decisions are influenced by an animal's experience with stimuli and rewards, with the goal of elucidating the underlying neural mechanisms of how this influencing occurs. We find that sensory-guided decisions can be rapidly biased by changes in the relative value of the two reward alternatives present in our task.

In a recent study, we examined the effect of changing the stimulus context on a rat's performance. We found that rats substantially improved their performance when a single difficult stimulus pair was presented repeatedly in a session compared to the standard condition in which the whole range of easy and difficult mixtures is interleaved. This effect reversed immediately when stimulus context was switched back, indicating a rapid and transient underlying plasticity. Interestingly, the performance gain was achieved without an increase in response time: There was no speed/accuracy tradeoff.

Dynamic Reinforcement-based Learning in Perceptual Decision Making

N. Uchida

Because environments can change dynamically, the ability to rapidly update behavioral strategies is crucial for survival. Recently, neuroscientists have begun to study adaptive decision making using statistical learning theory. This mathematical framework provides a theory for describing the optimal learning and execution of choice behavior. In particular, reinforcement learning models show how reinforcement history can be used to update a choice strategy to lead to a more adaptive strategy (i.e., policy). In these models, the role of uncertainty is critical. In an uncertain environment, i.e., one in which outcomes are hard to predict and prediction errors are large, adaptation may occur quickly. We tested how the uncertainty affected the performance of the odor mixture discrimination task by examining the effect of trial history on the performance. Strikingly, we found substantial effects of the recent previous experience of stimulus and reward. Animals were dynamically adjusting or updating their decision strategy even after extensive training. Essentially, what was happening was that rats biased their decisions to the recently rewarded direction, a so-called "win-stay" strategy. More surprisingly, this effect was much greater after rats were rewarded for an uncertain discrimination than for an easy discrimination. Thus, dynamic learning depended not only on history of reward but on uncertainty as well.

Control of Sniffing: Active Sensation and Olfactory Behavior

A. Kepecs, N. Uchida

Olfaction depends critically on an active motor process, sniffing, which draws air across the nasal epithelium. Respiration is proximally controlled by a central pattern generator (CPG) in the brain stem, but little is known about the central regulation of the respiratory rhythm during behavior.

Nasal thermocouples (small temperature-sensing probes) allow 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. We examine its frequency and phase in relation to odor sampling, and also to motor and reward events. Respiration frequency varied over a wide range (<2 Hz 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. Interestingly, rather than a continuous distribution of frequencies, discrete frequency modes were observed. Rats invariably switched to a theta frequency mode (8–10 Hz) shortly before (self-initiated) odor port entry and remained tightly locked to this frequency mode throughout odor presentation. The switch between different 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 CPGs, allowing both expectations to direct sensory acquisition as well as the coordination of respiratory and motor processes.

Single Unit Encoding of Goal-directed Behavior in Orbitofrontal Cortex

C. Feierstein, M. Quirk, N. Uchida

Every day we face the need to make decisions: We choose between different alternatives to achieve desired goals. 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. We are using chronic multi-electrode recording techniques to monitor the activity of ensembles of neurons in rats performing a two-alternative odor discrimination task (Fig. 1). Our studies have focused on the orbitofrontal cortex (OFC) and associated cortical and subcortical areas.

Orbitofrontal cortex is a region of the prefrontal cortex critical to incorporating predictions about expected outcomes (e.g., rewards) into decision making. Previous work shows that single OFC neurons differentially encode odors and visual stimuli according to associated rewards and punishments, but it is not known whether OFC neurons encode motor or response variables that would be required to learn the links between actions and their consequences or to make goal-directed choices. We find that a large fraction of OFC neurons are indeed tuned for response direction, either alone or in combination with stimulus or reward tuning. Analysis of error trials showed that single OFC neurons dynamically integrated olfactory (sensory) and spatial (response) information in a way that allows them to predict the outcome of a trial or to remember the stimulus associated with a delivered reward. As a population, OFC neurons are predominantly tuned for correct stimulus-response associations in task-trained animals and thereby correctly model the contingencies of the task. Our findings suggest that in the rat OFC, circuits not only link stimuli and reward but, like other areas of the prefrontal cortex, process information about the relationships between stimuli and actions.

Local Network Interactions in Prefrontal Microcircuits

M. Quirk, C. Feierstein

Although much is known about the cellular and synaptic components of cortical circuits, considerably less is known about how these circuits behave in the functioning brain. Using multi-electrode recording techniques, we are able to isolate action potentials arising from multiple cells within a small local volume of tissue (diameter <100 um). By applying these recordings to the prefrontal cortex of rats performing our two-alternative olfactory discrimination task (Fig. 1), we

are studying how information is represented within these microcircuits during the formation of a binary perceptual decision. In contrast to the typical columnar organization of primary sensory areas, even nearby cells display diverse response properties. The heterogeneity of responses across cells recorded in the same electrode was comparable to that of cells across electrodes, indicating that salient task variables (including stimulus, response, and reward information) represented in prefrontal cortex are available locally within individual microcolumns. To begin to characterize the functional architecture of a cortical microcircuit during a behavioral task, we analyzed pair-wise statistical interactions of the spike trains of simultaneously recorded cells. We find strongly enhanced interactions between neighboring neurons compared to distal neurons, suggesting that local inputs contribute to firing properties of cells. Interestingly, the spike trains of neighboring neurons are very often anticorrelated. The function of this inhibition seems to be twofold. First, inhibition between cells with opposite tuning properties promotes a form of "winner-take-all" competition essential for effective decision making. Second, inhibition between cells with similar tuning profiles provides a mechanism for effectively controlling the temporal flow of information within the circuit by limiting the number of cells active at a given moment in time. Together, these observations suggest a local circuit mechanism by which prefrontal cortex learns stimulus-response associations and uses them for decision making.

Neural Mechanisms of Context-dependent Decisions

D. Sosulski, M. Quirk

One of the biological foundations of cognitive flexibility—the ability to adapt one's behavior and thinking in the face of new or rapidly changing circumstances—is the brain's ability to integrate sensory information from several modalities, create an internal representation of the environment, and use this representation to subsequently guide its selection of behavioral response in a given situation. It has long been known that the prefrontal cortex is critical in situations when mappings exist between a single stimulus and a number of behavioral responses whose appropriateness depends on the current situation; however, the mechanisms by which the brain actually selects among possible behavioral responses remains unclear. To investigate the neural mechanisms by which stimulus-response association information and information about context interact in prefrontal cortex, we established a context-dependent decision-making task that requires rodents to respond in different ways to a stimulus, depending on the spatial context in which they experience it. In addition, we have recently started to use multi-electrode arrays to simultaneously record the activity of many single neurons in rats performing this context-dependent decision task. By analyzing how neurons that code for different stimulus-response associations are modulated by spatial context, we hope to gain some insight into how the brain integrates information about learned responses and the environment, and uses this information to successfully guide its behavior in a world that is continually in flux.

Inter-areal Coordination among Brain Regions during Olfactory Decisions

A. Kepecs

The brain has many interconnected areas specialized to carry out different functions. As with any complex network, it is essential for the brain to coordinate the activity of these many regions and to do so dynamically. 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?

Although there is no global clock, similar to those in computers, oscillations in neural activity have been widely observed at specific frequencies and in identified behavioral states. If large-scale coherent neural activity could be established across areas and dynamically controlled, specificity in neural communication could be achieved. This leads to the suggestion that transient and dynamic oscillatory synchronization between brain regions is used to differentially route and thus coordinate information flow. To examine this idea we are recording local field potentials (representing the summed coherent activity in local neuronal

populations) simultaneously from the olfactory bulb, olfactory cortex, and hippocampus in rats performing an olfactory discrimination task (Fig. 1). We are using spectral analysis to examine how features of the local field potentials correspond to different behavioral epochs and the nature of the relationship between oscillations in different regions. Strong theta (4–10 Hz) oscillations appear at all sites during the task, whereas beta (15–30 Hz) frequency oscillations are transient, occurring specifically around the time of choice in the olfactory bulb and ventral hippocampus. Interestingly, the ventral hippocampus shows differential coordination with different anatomical partners: theta band coherence with the dorsal hippocampus and transient beta band coherence with the olfactory bulb. These findings are beginning to paint a broad-scale picture of the spatiotemporal organization of interareal coordination during olfactory discrimination.

Olfactory Discrimination in Mice

S. Ranade, N. Uchida

We have developed and extensively characterized the behavior of rats during the performance of a two-alternative olfactory discrimination task (Fig. 1). However, to harness the power of transgenic gene manipulation technologies for use in the study of the neural basis of decision making and other cognitive functions, it is important to develop behavioral paradigms suitable for use with mice, for which many genetic models and manipulations currently exist. With this goal in mind, we have trained mice to perform a two-alternative olfactory discrimination task. We find that, similar to rats, mice can perform this task with high accuracy (>95% total score) and they require only 4–6 sessions (~1000 trials) to reach criterion performance (>85%). Performance remains stable over days and even months. In addition, the reaction time (odor sampling time) of mice performing the two-alternative task is very similar to rats (~300 ms). Using this well-controlled behavioral task we can explore different aspects of brain function from sensation to action. In collaboration with Karel Svoboda's group here at CSHL, we are applying transgenic technologies to inducibly inhibit neural activity in restricted populations of olfactory sensory neurons. This approach will enable us to examine the effect of specific manipulations of sensory input on an animal's perception as assayed by his performance.

Neural and Metabolic Activity in Olfactory Glomeruli

H. Gurden, S. Ranade, N. Uchida

Neural activity is mirrored by metabolic activity, which in turn causes physiological signals that can be detected using functional imaging techniques. The mechanisms that link these processes are the subject of intense interest. We are investigating the link between odor-evoked neural activity and intrinsic optical signals (IOS) in the olfactory glomeruli. IOS are used for studying the representation of odors, but we hope to clarify how to interpret IOS as a readout of glomerular activity and the mechanisms that link neural activity to metabolism and functional imaging signals.

We used in vivo imaging and simultaneous local pharmacology to study how sensory-evoked neural activity is transduced into IOS in the well-defined circuitry of the olfactory glomerulus. Odor-evoked IOS were tightly coupled to release of glutamate and were strongly modulated by exogenous and endogenous activation of presynaptic dopamine and γ-amino-*n*-butyric acid (GABA)-B receptors. Surprisingly, IOS were independent of postsynaptic transmission through ionotropic glutamate receptors. Instead, IOS depended on the activation of astrocytic glutamate transporters. Thus, glutamate uptake by astrocytes is an important pathway by which neural activity is coupled to functional imaging signals and through which metabolic and information processing networks share information in the brain.

Calcium Dynamics Underlying Dendritic Integration in Olfactory Bulb Granule Cells

V. Egger [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory]

In the mammalian olfactory bulb, axonless granule cells process synaptic input and output reciprocally within large spines. The nature of the calcium signals that underlie the pre- and postsynaptic function of these spines is largely unknown. Using two-photon imaging in acute rat brain slices and glomerular stimulation of mitral/tufted cells, we observed two forms of action potential-independent synaptic Ca^{2+} signals in granule cell dendrites. Weak activation of mitral/

tufted cells produced stochastic Ca^{2+} transients in individual granule cell spines. These transients were strictly localized to the spine head, indicating a local passive boosting or spine spike. Ca^{2+} sources for these local synaptic events included NMDA receptors, voltage-dependent calcium channels and Ca^{2+}-induced Ca^{2+} release from internal stores. Stronger activation of mitral/tufted cells produced a low-threshold Ca^{2+} spike (LTS) throughout the granule cell apical dendrite. This global spike was mediated by T-type Ca^{2+} channels and represents a candidate mechanism for subthreshold lateral inhibition in the olfactory bulb. The coincidence of local input and LTS in a spine resulted in summation of local and global Ca^{2+} signals, a dendritic computation that could endow granule cells with subthreshold associative plasticity.

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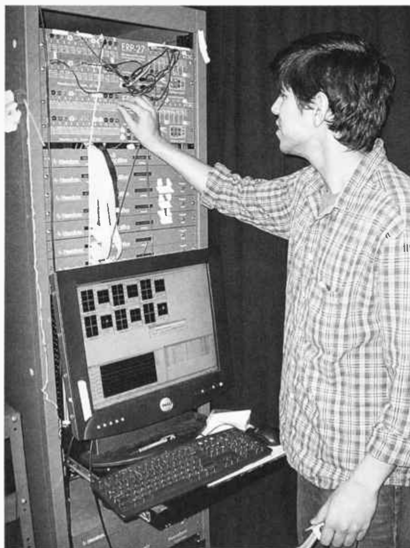

Sachin Ranade, grad student, Zach Mainen's lab.

TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow A. Barria, J. Boehm, I. Ehrlich, R. Huganir, H.W.H.G. Kessels, M. Klein, C. Kopec, J. LeDoux, B. Li, S. Rumpel, W. Wei

This laboratory is directed toward an understanding of 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-methyl-3-isoazole (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors at synapses that may underlie plasticity and the malfunction that could lead to diseases. Some of our studies our summarized below.

Postsynaptic Receptor Trafficking Underlying a Form of Associative Learning

S. Rumpel, J. LeDoux, R. Malinow [in collaboration with A. Zador, CSHL]

To elucidate the 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. 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 for this learning process. Furthermore, memory was reduced if AMPA receptor (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.

NMDA-R Subunit Composition Controls Synaptic Plasticity by Regulating Binding to CaMKII

A. Barria, R. Malinow

Calcium entry through postsynaptic NMDA-Rs and subsequent activation of calcium/calmodulin-dependent protein kinase II (CaMKII) are events that trigger synaptic plasticity in many brain regions. Upon activation, CaMKII can associate with NMDA-Rs, but the physiological role of this interaction is not well-understood. In this study, we tested if association between active CaMKII and synaptic NMDA-Rs is required for synaptic plasticity. We used organotypic hippocampal slices in which long-term potentiation (LTP) requires CaMKII activity and NR2B, an NMDA-R subunit that binds with high affinity to CaMKII. We found that switching synaptic NR2B-containing NMDA-Rs with those containing NR2A, a subunit that shows low affinity for CaMKII, dramatically reduces LTP. Expression of NR2A with mutations that increase association to active CaMKII recovered LTP. Finally, driving into synapses NR2B with mutations that reduce association to active CaMKII prevented LTP. We conclude that association between active CaMKII and NR2B is required for hippocampal LTP in this early developmental period. The switch from NR2B to NR2A content in synaptic NMDA-Rs normally observed in many brain regions may control plasticity by reducing the binding of active CaMKII.

Spine Enlargement Precedes AMPA-R 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-Rs onto dendritic spines. In contrast, the same stimulus produced a small reduction of NMDA-Rs from spines. LTP produced a similar modification of small and large spines. Interestingly, during LTP induction, spines increased in volume before surface incorpora-

tion of AMPA-Rs, indicating that distinct mechanisms underlie changes in morphology and receptor content.

Differential Subcellular Overexpression of AMPA-R Subunits in CA1 Neurons

H.W.H.G. 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 although 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.

Direct Phosphorylation by PKC at a Novel Site on an AMPA-R Subunit GluR1 Controls Synaptic Incorporation during LTP

J. Boehm, R. Huganir, R. Malinow

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 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.

PSD-95 Controls AMPA-R Incorporation during Long-term Potentiation and Experience-driven Synaptic Plasticity

I. Ehrlich, R. Malinow

The regulated delivery of AMPA-type glutamate receptors to synapses is an important mechanism underlying synaptic plasticity. Here we ask if the synaptic scaffolding protein PSD-95 participates in AMPA-R incorporation during two forms of synaptic plasticity. In hippocampal slice cultures, expression of PSD-95-GFP increases AMPA-R currents by selectively delivering GluR1-containing receptors to synapses, thus mimicking LTP. Mutational analysis shows that the amino terminus of PSD-95 (including the first two PDZ domains) is necessary and sufficient to mediate this effect. Further supporting a role in synaptic plasticity, wild-type PSD-95 occludes LTP, and dominant-negative forms block LTP. Moreover, we demonstrate that PSD-95 participates also in AMPA-R delivery during experience-driven plasticity in vivo. In barrel cortex from experience-deprived animals, expression of PSD-95-GFP selectively increases AMPA-R currents, mimicking experience-driven plasticity. In nondeprived animals, PSD-95-GFP produces no further potentiation, indicating common mechanisms between PSD-95-mediated potentiation and experience-driven synaptic strengthening. A dominant-negative form of PSD-95 blocks experience-driven potentiation of synapses. Pharmacological analysis in slice cultures reveals that PSD-95 acts downstream from other signaling pathways involved in LTP. We conclude that PSD-95 controls activity-dependent AMPA-R incorporation at synapses via PDZ interactions not only during LTP in vitro, but also during experience-driven synaptic strengthening by natural stimuli in vivo.

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INTEGRATIVE SYSTEMS NEUROBIOLOGY

P.P. Mitra P. Andrews
H. Bokil
H. Maniar

Partha Mitra (previously located at Bell Laboratories, Lucent Technologies) joined the faculty at CSHL in the fall of 2003 when this research effort began. Hemant Bokil is a postdoctoral fellow continuing his work on signal processing applications to neurobiological time series with special emphasis on neural prostheses. Hiren Maniar, a postdoctoral fellow, is working as part of a consortium funded by the Human Frontiers Science Foundation on the role of neural synchrony in integration across sensory modalities. Peter Andrews joined the group in August 2004 after finishing his Ph.D. in physics and a postdoc at Yale University, where he was responsible for the programming and data analysis for the QUEST quasar survey. Peter is performing the dual role of providing software infrastructure for the group, as well as participating in the open source software project *Chronux* to develop software for neurobiological data analysis.

Apart from this group of core personnel, our research effort includes close collaborative ties with multiple research groups both locally and globally. A collaborative effort with Niko Schiff and Keith Purpura at the Weill Medical School of Cornell University, where Partha is an adjunct associate professor, involves electrophysiology in the awake behaving macaque. A second research effort on how zebra finches learn their song vocalizations is being carried out in collaboration with Ofer Tchernichovski at City College of New York. Recently, this research effort has been broadened into a consortium of zebra finch researchers based in four different universities. A third collaboration is with the group of Rodolfo Llinas 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 (California Institute of Technology), Pascal Fries (F.C. Donders Research Center at Nijemegen), Shiny'a Nishida at the Nippon Telegraph and Telephone Corporation Labs in Japan, and Alan Johnston at University College, London in the United Kingdom.

Our basic research philosophy is to bring computational and theoretical tools to bear on biological questions in general and neurobiological questions in particular, to make sense of biological complexity and to provide understanding and potential therapies for disease. There are two basic branches to the research: neuroinformatics and theoretical engineering. The former involves the application of statistical and computational tools to neurobiological data, primarily single-channel and multichannel time series, including point processes (spike trains) and brain imaging data. The applications are geared toward exploratory analysis of large volumes of data, as well as confirmatory analysis for testing of specific hypotheses. This is a continuing research effort that reflects several years of work and is associated with a course entitled "Neuroinformatics" held each summer at the Marine Biological Laboratories in Massachusetts. The second branch of research deals with the question of theoretical principles underlying the design of biological systems. Since biological organisms are evolved to perform their tasks robustly under uncertain circumstance, in terms of both the environment and parametric uncertainty in components making up the system, it is expected that the mathematical theories developed by engineers to help design man-made systems that perform under similarly challenging circumstances may also apply to these systems. The goal of this research is to identify the engineering principles most germane to biology and to study selected examples drawn from different levels of the organizational hierarchy.

Chronux: Open Source Software for Analyzing Neurobiological Time Series Data

H. Bokil, P. Andrews

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 electri-

cally to large groups of neurons monitored indirectly through a variety of imaging methodologies. The relevant measurements consist of single or multichannel time series data, in the form of continuous or point processes. Therefore, the statistical analysis of time series data is central to studying the nervous system.

During the last several years, our research has involved the development of algorithmic and computational tools for the analysis of multichannel time series data from the neurosciences. We are now encoding these tools into an open source software package entitled *Chronux*. This project will proceed in stages and involve the development of a high-quality numerical analysis library, data IO and management utilities, and a user interface that provides experimental neuroscientists access to advanced analysis tools. The project will proceed in stages, where we move from the current implementation of the relevant routines as MATLAB toolboxes, to a fully open source and self-contained software. Although there are a number of signal processing libraries available, the goal of this effort is to develop domain-specific tools that will aid neuroscience research. We expect that tools such as these will be critical to advancing our understanding of systems neuroscience.

The past year has seen substantial improvements made to *Chronux*. On the algorithmic side, we have added routines that provide robust estimates of firing rates based on local regression methods. We have also created the *Chronux* "knowledge environment," which is a Web-based tool for documenting *Chronux* and collaboratively working on algorithm development. We continue to work on developing new statistical tools for time series analysis, and recent advances include extensions of some standard measures from spectral analysis to particular cases relevant to neurobiological data analysis. We have recently presented a review of the growing field of neural time series analysis (Brown et al. 2004).

Temporal Structure in Neural Activity during Working Memory

P.P. Mitra [in collaboration with K. Purpura and N. Schiff, Weill Medical School, Cornell University]

Holding items in short-term memory is thought to correspond to appropriate holding patterns of electrical activity in the brain. An experimental model system much studied in this regard involves memory saccades in awake behaving macaques, where the subject remembers a target location during a memory period and performs a visual saccade to the target on an appropriate cue. Area LIP, on the lateral bank of the intraparietal sulcus, is known to encode intended saccade directions. Individual neurons may be found in this area which show a transiently elevated firing rate during the memory period of a saccade memory task, with tuning to the direction of the intended saccade.

In collaborative work with Richard Andersen's lab at Caltech, we have previously discovered that in addition to the firing rates, the dynamics of the neural activity, as reflected in spectral measures, also shows tuning to intended saccade direction. This project has been transferred to the Weill Medical School of Cornell University, where it is being carried out in collaboration with K. Purpura and N. Schiff. In the previous year, we completed the setup phase of the new laboratory and began recording from one monkey with dual chambers over the parietal and frontal cortices. Preliminary recordings from the frontal eye fields show behaviorally tuned spectral structure in the neural activity, analogous to the original findings for recordings from area LIP. These results were presented at the annual Society for Neuroscience meeting (2004).

Song Learning in the Zebra Finch

P.P. Mitra [in collaboration with O. Tchernichovski, City College of New York]

The neural substrate and evolutionary origins of linguistic behavior in humans is of great biological interest, but difficult to study, since there are no other species where the acoustic communication behavior is seen to exhibit the same level of syntactic complexity. Therefore, there has been great interest in studying what has been termed the faculty of language in a broad sense, one aspect of which is the ability to learn vocalizations. This last is itself infrequently found in animals—a reason why songbirds, which exhibit learning of complex vocal repertoires, have been the subject of intensive study. The species of choice in this research is the zebra finch, where all aspects of the song system have been scrutinized, ranging from the sensory motor circuitry in the brain to the production apparatus. We have developed techniques for detailed quantification of song development, through the analysis of continuously acquired acoustic signals from birds subjected to a controlled training protocol.

In this ongoing research project, Sebastien Dergnaucourt, a postdoc in the Tchernichovski laboratory, has demonstrated that sleep affects song learning, particularly in the early stages: It appears that song performance degrades after night sleep during the first few weeks after exposure to the song model. This "morning deterioration" is absent in the adult bird. Somewhat counterintuitively, the birds that show the largest morning deterioration also show the best final imitation. Although we do not yet fully understand the mechanistic basis of this effect, we have demonstrated that it is directly related to sleep: The effect was seen to occur after induction of sleep using melatonin. It is known that the zebra finch brain exhibits a replay of the neural patterns associated with song during sleep, leading to the hypothesis that the observed deterioration reflects an active process that occurs during sleep. This work was recently reported in *Nature* (Deregnaucourt et al. 2005).

Vocal Development in Infants

P.P. Mitra

Given the success of the extensive longitudinal recordings of song from the developing zebra finch, we have started a study of human vocal development. The idea is to carry out longitudinal recordings of infant vocalizations over an extended period and to analyze the resultant data to quantify the vocal development. Similar studies in the past have relied on human transcriptions of infant sounds, or sparse recordings. The current study will increase the coverage in terms of recordings and will develop automated tools for analysis and quantification.

Source Localization of Neural Activity in MEG and EEG

H. Maniar, P.P. Mitra

Noninvasive imaging techniques have a critical role in studies pertaining to cognitive processes and in determining pathological conditions in the human brain. Ideally, the imaging technique would concurrently exhibit both high temporal and spatial resolution. Techniques based on hemodynamic changes, such as PET, SPECT, and especially fMRI, have a relatively high spatial resolution but poor temporal resolution. In contrast, techniques measuring neural electrical activity such as MEG and EEG have high temporal resolution, but a spatial resolution dependent on the inverse methodology. We have been working on the computational implementation of a new inverse methodology, entitled Local Basis Expansions, for localization of neural activity. This work is part of the Human Frontiers Science Program consortium effort with Shinsuke Shimojo, Pascal Fries, and others, where our methods will be employed to localize broadband oscillatory activity in MEG and EEG measurements. The tool will eventually be made part of the *Chronux* platform.

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EXPERIENCE-DEPENDENT PLASTICITY OF SYNAPSES AND SYNAPTIC CIRCUITS

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

The functional properties of the brain must change in response to salient sensory experiences. The nature of these changes at the level of synapses, neurons, and their networks, also known as the engram, is unknown. 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. 2-photon laser-scanning microscopy (2PLSM) allows us to image single synapses in intact tissues and to track changes in intracellular calcium and signal transduction events. Excitation of neuronal elements by focal uncaging of neurotransmitters allows us to probe the connectivity of neural networks with high efficiency. We combine these optical methods with electrophysiological measurements of synaptic currents and potentials and molecular manipulations of neurons.

We use both in vivo measurements to address system level questions and in vitro methods to get at detailed mechanisms. As a model system, we use the rodent barrel cortex, 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.

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

V. Scheuss, A. Sobzcyk

Long-term changes in synaptic efficacy are triggered by *N*-methyl-D-aspartate (NMDA) receptor-mediated increases in [Ca^{2+}] in the postsynaptic neuron. We have developed a variety of assays to learn about the molecular pathways of Ca^{2+} influx, including the counting of individual Ca^{2+} permeable channels and receptors in spines. W have recently discovered that different spines are preferentially populated with different types of NMDA receptors (NMDA-Rs) (NR2A vs. NR2B). Interestingly, these different types of receptors have different Ca^{2+} permeabilities, indicating that NMDA-Rs in synapses have the ability to regulate their Ca^{2+} permeability independent of their conductance.

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

R. Yasuda, C. Harvey, 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. Fluorescence lifetime imaging microscopy (FLIM) allows us to image 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.

Experience-dependent Plasticity in the Adult Cortex In Vivo

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

Sensory representations in the adult brain are stable, yet we are able to learn. Which neural elements are plastic in the neocortex, especially in response to novel sensory experience? Answers to these questions are fundamental to the mechanisms of plasticity and the memory capacity of the brain. Our approach has

been to image structural dynamics in vivo. A breakthrough was the development of long-term (months) time-lapse imaging at the level of individual synapses in the *adult cortex* of transgenic mice together with retrospective serial-section electron microscopy.

We find that the large-scale arborization 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. Plasticity differs among cell types. We have focused on understanding the plasticity of L5B neurons. L5B spines consist of two distinct populations: small "transient" spines that live for days and large "persistent" spines that live much longer (months). Under baseline conditions, at least half of the transient spines have synapses. Induction of plasticity by trimming a subset of whiskers transiently (~1–4 days) increases spine turnover, followed by stabilization (after ~1 week) with new persistent synapses.

Circuit Mechanisms of Experience-dependent Plasticity

G. Shepherd, I. Bureau, 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 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 neurons. We discovered "septal" columns between the "barrel" columns. These columnar systems are part of parallel thalamocortical circuits. 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. What is the cellular basis for the differences between these projections? The synaptic weight per synapse? The density of synapses per wire length? The fraction of connected neurons or the number of synapses between connected neurons? To address these and related questions, we will develop novel approaches for circuit mapping with single-cell resolution.

Also using LSPS, we measured the functional and anatomical development of cortical columns, finding remarkable specificity, without detectable "overgrowth and pruning." We further performed an unbiased search for experience-dependent synaptic pathways during development. The strengths of excitatory L4→L2/3 connections changed with opposing signs in barrel and septal columns, explaining previously observed receptive field changes. The identification of these pathways is allowing us to determine the cellular mechanisms of experience-dependent synaptic plasticity.

Development of Thalamocortical Axons

R. Weimer [in collaboration with C. Portera-Cailliau, University of California, Los Angeles]

The development of mammalian cortical axons has been studied extensively in histological preparations. However, important questions about axonal development require time-lapse imaging. What is the balance between growth and pruning? How rapid are growth and pruning? Do all axons develop with similar sequences or are there multiple strategies for axon elaboration? To address these issues, we have used 2-photon time-lapse microscopy to image axons in GFP (green fluorescent protein) transgenic mice during the first 3 weeks of postnatal development. This period spans the arrival of thalamocortical (TC) axon collaterals to layer 1, the presence and disappearance of Cajal-Retzius (CR) neurons, cortical neuronal migration, and barrel cortex critical periods. The elaboration of local axonal arbors relies on a careful balance of growth and pruning of axon segments that favors overall growth only slightly. Pruning involved either retraction of small axon segments (tens of micrometers) or fragmentation of much larger branches (hundreds of micrometers). The dynamics of axonal arbors of CR and TC neurons differed profoundly. TC axonal branch tips lack distinct growth cones and grow rapidly in straight paths, with frequent intersitial branch additions. The axon tips of CR axons have large growth cones with numerous long filopodia and grow slowly following tortuous paths. Given the identical terrain upon which CR and TC axons grow, these two distinct modes of growth likely reflect different intrinsic growth programs for long-range projection neurons versus local interneurons.

Cellular Mechanisms of Synapse Development

K. Zito

Most of the excitatory synapses in the cortex occur on dendritic spines, small protrusions extending from neuronal dendrites. To determine if spine growth is associated with synapse formation, we studied Neurabin-I (NrbI), a *neur*onal specific *a*ctin-*bin*ding protein. A severely truncated GFP-NrbI caused a dramatic increase (more than twofold) in the number of spines. Using electron microscopy, we found that Nrb is concentrated at sites of cell-cell contact and that morphological changes reflect an increase in the number of synapses. Nrb truncations drove the polymerization of spine actin to favor spine growth and synapse formation.

Molecular Methods to Reversibly Inactivate Synapses In Vivo

A. Karpova, G. Tervo, 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 *m*olecules for *i*nactivation of *s*ynaptic *t*ransmission (MISTs) that can genetically be 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.

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NEUROGENETICS OF MEMORY IN *DROSOPHILA*

T. Tully J. Barditch Y. Hua D. Leong M. Regulski
F. Bolduc K. Iijima-Ando J. Luo N. Sinha
L. Grady J. Kui C. Margulies S. Xia

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 CREB and cAMP signaling, *staufen*, and regulation of local protein translation.

We have established a role for *N*-methyl-D-aspartate (NMDA) receptors (NMDA-Rs) in *Drosophila* learning and long-term memory (LTM) formation. The *Drosophila* genome carries homologs of vertebrates *NR1* and *NR2*, subunits of which must coassemble to form functional NMDA channels in vertebrates. Similarly, *dNR1* and *dNR2* must be coexpressed to form voltage-dependent NMDA-responsive channels in flies (see Fig. 1). Flies homozygous for either of two P-element insertion mutations of *dNR1* display moderate defects in olfactory associative learning, which can be rescued via transgenic expression of wild-type *dNR1*. Acute expression of anti-RNA for *dNR1* in adult flies disrupts 1-day memory after spaced but not massed training, indicating a specific disruption of CREB-dependent LTM. NR1 and NR2 appear to be expressed widely throughout the adult brain, with preferential expression in a few novel neurons. These data demonstrate an evolutionarily conserved role for NMDA-Rs in associative learning and memory.

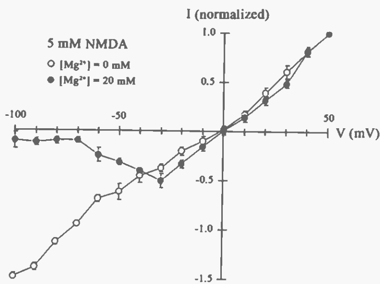

FIGURE 1 *Drosophila* NMDA-R function is voltage dependent in the presence of magnesium, a key property of vertebrate NMDA-Rs.

MUTANTS AND MICROARRAYS

More recent investigations of NMDA-R function in *Drosophila* have been accomplished by engineering transgenic flies carrying an upstream activation sequence (UAS)-driven anti-NR1 RNAi (RNA interference) transgene. We have crossed these transgenic flies with MB (mushroom body) GAL4 drivers to determine the role of NMDA-Rs in MBs. Preliminary data suggest that acute disruption (using *hs-GAL4*) of NR1 in this manner yields defects specific to middle-term memory (MTM), but does not affect learning or LTM.

We continue experiments using DNA microarrays to detect gene expression changes associated with LTM formation. To date, we have done follow-up Q-PCR (quantitative polymerase chain reaction) on 137 candidate memory genes (CMGs) and have confirmed 51, yielding a true-positive "hit rate" of 37%. Our ultimate goal is to provide in vivo validation that these confirmed CMGs are involved in adult memory formation. To this end, we have developed a method to screen short interfering RNA (siRNA) sequences for target genes in cultured S2 cells, using branched-DNA methodology. Foldback RNAi transgenes are then constructed with a DNA sequence surrounding the effective siRNA sequence. These foldback RNAi constructs are cloned into a UAS-driven vector, which we have constructed to optimize the expression of these RNAi constructs in vivo. So far, we have generated transgenic flies for NR1 (a positive control for our UAS-driven vector system) and have identified effective siRNA sequences for *slmb*, *aur*, and *thor*. Transgenic flies carrying RNAi constructs for these genes are cur-

rently being generated and will be induced in adults (using *hs-GAL4*) to assess an acute role for each CMG in memory formation.

In collaboration with Gengxin Chen in Michael Zhang's lab and Qing-Shuo Zhang in Adrian Krainer's lab here at CSHL, we have developed a bioinformatics approach to identify candidate genes carrying a nanos response element (NRE) in their mRNA transcripts, to which PUMILIO likely binds. On the basis of our previously published work, CMGs that also carry NREs are likely to represent genes, the transcripts of which are involved in the local control of protein translation during LTM formation. This approach serves to expand the *pumilio/staufen* pathway to include additional genes and molecular mechanisms.

In collaboration with Dr. Yi Zhong and co-workers at CSHL, we have shown that *Notch* functions during LTM formation in *Drosophila*. Interestingly, overexpression of *Notch* leads to enhanced memory formation in a manner similar to that for overexpression of the CREB activator (see Publications below). These observations again identify additional genes that may impinge on the *pumilio/staufen* pathway.

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 UAS-driven expression of a CREB-repressor transgene, using several different MB GAL4 drivers, does NOT block LTM formation. Conversely, we have identified one enhancer GAL4 line driving expression of UAS-CREB-r (repressor) in central complex that does block spaced training-specific 1-day memory (LTM). We are currently characterizing additional enhancer GAL4 drivers with overlapping expression in central complex, including some of the enhancer GAL4 lines identified by us as memory mutants.

We have developed a new method to cell-sort GFP (green fluorescent protein)-expressing neurons from dissociated adult brains. To maximize the protocol, we expressed GFP in MBs, sorting dissociated cells into GFP$^+$ versus GFP subsets. Under these conditions, Q-PCR reveals a 512-fold higher GFP expression in GFP$^+$ versus GFP cells. Q-PCR-derived expression of CREB, Adf1, fasII, and rut were –.8-, –.5-, 8-, and 2-fold higher in GFP$^+$ versus GFP cells. On the basis of published immunostaining observations, we expect to observe no differences in expression for CREB and Adf1 and preferential expression of fasII and rut in MB cells. We are currently repeating and extending these results and perfecting our cell-sorting method. If proven reliable, this new methodology will allow important new molecular and biochemical experiments on cell types identified to be involved in different aspects of memory formation (see above).

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 this learning deficit can be rescued by transgenic expression of a genomic construct. We are currently evaluating an acute role for *FMR1* in memory formation.

We also have begun to study a *Drosophila* model of Parkinson's disease. In many vertebrate manifestations of this form of heritable neurodegeneration, neurons overexpress α-synuclein. Similarly, transgenic overexpression of α-synuclein yields neurodegeneration in flies. Accordingly, we have begun DNA microarray studies in cultured *Drosophila* cells overexpressing α-synuclein to identify candidate genes involved in this form of neurodegeneration.

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NEURAL SUBSTRATE OF SELECTIVE AUDITORY ATTENTION IN THE CORTEX

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G. Otazu M. Wehr

We use a combination of theoretical and experimental approaches to study how the brain computes. The brain is able to solve hard computations that remain far beyond the reach of the fastest computers. Our goal is to understand this computation at the synaptic, cellular, behavioral, and algorithmic levels.

One example of such a hard computation is the "cocktail party problem." When we tune in to one voice at a cocktail party, and tune out the others—a task that remains beyond the capacity of modern computers—we are engaging in a form of selective auditory attention. Our ability to attend selectively is not limited to the auditory domain: Analogous tasks demand selective attention in the visual and even somatosensory domains. In monkeys, visual attention selectively enhances neural activity even in the earliest stages of visual cortical processing. This enhancement is surprising because the areas associated with these first stages of visual processing have traditionally been thought of as representing the sensory world faithfully, in a way that depends only on the properties of the sensory input itself. The discovery of attentional modulation overturns the notion that the peripheral sensory cortex is a passive "TV screen" available for viewing by a "homunculus" buried deep within the cortex.

The specific projects in our laboratory fall into two main categories. First, we are interested in how neurons represent auditory stimuli, and how these representations are computed from the cochlear inputs half a dozen synapses away. To address these questions, we are using electrophysiological and imaging approaches in anesthetized rats, as well as computational approaches, to characterize the properties of natural sounds. Second, we are interested in how these representations are modified dynamically—within seconds 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; but for other neurons, "shared" sources of variability produce circuit-wide fluctuations in the synaptic drive to the neuron and its neighbors, greatly increasing the apparent noise in the PSP. These stimulus-independent correlations could provide a substrate for feedback underlying cognitive processes, such as attention and motivation.

Sparse Synchronous Inputs Drive Neurons in 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 dynamics of one neuron's spiking output must be consistent with the ensemble activity of the population of neu-

rons 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, based on 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 a multi-electrode 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 AMPA (α-amino-3-hydroxy-5-methyl-4-isoazole) receptors 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 the 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 is reduced if AMPA receptor synaptic incorporation is 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 are currently extending these results to the auditory cortex.

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.

Sparse Representations for the Cocktail Party Problem

H. Asari [in collaboration with B. Pearlmutter, National University of Ireland, Maynooth]

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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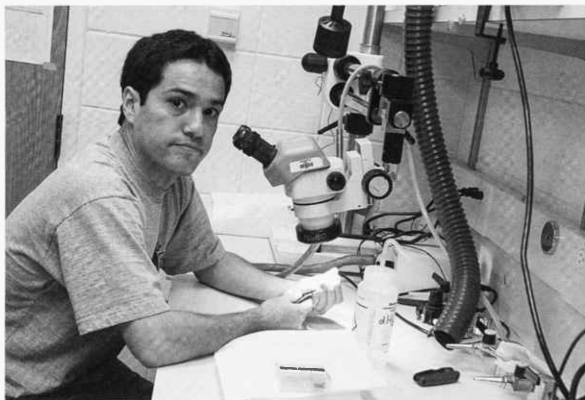

Gonzalo Hugo Otazu Aldana, postdoc, Anthony Zador's lab.

NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong H.-F. Guo K. Iijima
I. Hakker A. Mamiya
F. Hannan Y. Wang
I.S. Ho

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 interesting in genes known to contribute to neurofibromatosis 1 (*Nf1*) and Alzheimer's disease. *Nf1* patients are identified by neurofibromas and other symptoms including learning defects. We are investigating a hypothesis that the tumor-suppressor gene *Nf1* not only acts as a Ras-specific GTPase-activating protein (GAP), but also is involved in mediating G protein- and Ras-stimulated activation of adenylyl cyclase (AC); NF1-dependent AC pathways are required for learning and memory. 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 accumulation of Aβ deposits. We are now attempting to understand the molecular basis for Aβ toxicity. Second, we are investigating how odors are encoded by population neuronal activity in the fly brain and will ultimately study learning and memory at the level of population neural activity. Such study is carried out in living flies via optical recordings. The specific projects are described below.

Genetic Dissection of NF1-regulated Pathways in *Drosophila*

F. Hannan, I.S. Ho, I. Hakker

NF1 is an inherited disorder that affects 1 in 3500 people worldwide. A large number of different mutations are seen in NF1 patients, including some changes that only alter a single amino acid out of the whole NF1 protein, yet still result in NF1 disease. Several of these missense mutations have been constructed in our lab and introduced into *Nf1* mutant *Drosophila*. These mutated human NF1s can overcome some cAMP-dependent defects in *Nf1* mutant flies, including small body size and learning defects, indicating that the cAMP pathway is not affected by these mutations.

Two mutations in the GAP-related domain (GRD), however, show defective long-term memory even though learning is normal in these flies. This suggests that learning and memory may be differentially regulated in flies by NF1 acting through cAMP and Ras pathways, respectively. Verification of this hypothesis will provide important insights into the mechanism whereby NF1 affects learning. We propose to generate more missense mutants and some large deletion mutants, to look at their effects in *Nf1* mutant flies, to try to pinpoint the region(s) associated with cAMP activity versus Ras activity.

We have also recently identified a novel pathway for NF1-dependent AC activation in flies that involves growth factor stimulation of Ras. This pathway requires both Ras and NF1 activity to stimulate cAMP production. A second pathway requiring NF1 (but not Ras) activates the rutabaga AC which is critical for learning in flies. The mutant human NF1s provide an important resource for studying the role of NF1 in this new pathway because many other components of the pathway cannot be fully knocked out in flies since they have lethal effects.

A *Drosophila* Model of Alzheimer's Disease

K. Iijima

Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by the progressive decline in memory accompanied by histological changes, including neuronal loss and the formation of neurofibrillary tangles (NFTs) and senile plaques (SPs). The accumulation of $A\beta_{42}$ peptide, the major

component of SPs, has been hypothesized as the primary event in AD pathogenesis. Multiple pathogenic mutations have been identified in β-amyloid precursor protein (APP), Presenilin1 (PS1), and Presenilin2 (PS2) genes in familial AD (FAD) and indicated that all of these mutations caused excessive accumulation of $A\beta_{42}$. Therefore, decreasing the level of $A\beta_{42}$ as well as its toxicity in the brain would be one of the most promising strategies among mechanism-based therapy for AD. However, little is known about the molecular mechanisms underlying memory defects and neurodegeneration caused by $A\beta_{42}$.

To address this question at animal level, we used the fruit fly *Drosophila* as a model organism. 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 in existing mouse AD models. This study strongly supports the idea that excessive accumulation of $A\beta_{42}$ is sufficient to cause memory defects and neuronal cell death resembling AD and suggests that the molecular basis underlying the $A\beta_{42}$ toxicity can be conserved in the fruit fly. We are using this fly AD model to conduct several approaches to understand the molecular and cellular mechanisms underlying $A\beta_{42}$-induced memory defects and neurodegeneration.

Visualization of Olfactory Learning and Memory in Mushroom Bodies

Y. Wang, A. Mamiya

The *Drosophila* mushroom body (MB) is a higher brain center that is crucial to associative 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 MB intrinsic neurons, also called Kenyon cells (KCs), in which axons are bundled separately into respective α/β, α′/β′, and γ lobes. Using a green fluorescent protein (GFP)-based Ca^{2+} sensor, G-CaMP, we have attempted to visualize learning-and-memory-associated changes in neuronal activities in the MB. Two different systems have been employed: an immobilized living fly and an isolated brain. In the living fly system, MB neuronal activities are monitored while the fly is being trained and tested for learning and memory. The training follows a classical Pavlovian paradigm, in which odor stimulation is given to the fly paired with electric shock to its legs. Neuronal activities in the MB are imaged through a tiny window cut in the fly head. In the isolated fly brain system, the aversive olfactory learning is mimicked by paired stimulation of the antennal nerve (AN) and the ventral nerve cord (VNC). This system also allows mimicking association between olfaction and other sensory modalities, such as visual and taste stimulations.

After pairing of the stimulation of the AN and VNC, activities in the γ and β′ lobes are enhanced in response to stimulation of the AN alone. This enhancement can last up to 1 hour. Multiple sessions of paired stimulation of the AN and VNC with an interval of 5–10 minutes lead to significant enhancement of activities in the α′ lobe in response to stimulation of the AN alone. Our finding is consistent with a behavioral study which suggests that the α and α′ lobes are involved in long-term memory, whereas the γ lobe is required for short-term memory.

Role of Akt in Synaptic Plasticity and Learning

H.-F. Guo

Long-term modification of synaptic strength is important for refining neuronal circuits and is believed to be the cellular substrate for learning and memory. The *Drosophila* larval neuromuscular junction (NMJ) is a well-established preparation enabling quantitative analyses of synaptic physiology at identifiable synapses. Various forms of short-term synaptic plasticity have been demonstrated at the *Drosophila* NMJ, but long-term plasticity such as long-term potentiation (LTP) and long-term depression (LTD) has not been shown. Identification and characterization at the *Drosophila* NMJ would enable the use of powerful *Drosophila* genetic tools for ana-

lyzing long-term plasticity and provide additional function assays for studying functions of learning and memory genes.

We have extensively explored long-term plasticity at the *Drosophila* NMJ. To this end, we have identified and characterized synaptic LTD at these glutamatergic synapses. We found that applying tetanic stimulation (tetanus) of defined frequency and duration can reliably induce robust LTD. The level of depression is dependent on tetanus frequency and Ca^{2+} concentration. The LTD is primarily due to presynaptic changes. Genetic analysis indicates that Akt is essential for LTD, which is greatly impaired in *akt* mutants, and the impairment was rescued by acutely induced expression of the *akt* transgene. In addition, preliminary data suggest that Akt is also important for *Drosophila* olfactory learning.

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Inessa Hakker, lab tech, Yi Zhong's lab.

PLANT DEVELOPMENT AND GENETICS

From roots and stems to leaves and flowers, plants have evolved into an astonishing variety of forms tailored by their ecological niches. Exploring the genetic programs that enable plants to develop from seed to maturity reveals information that not only advances biological knowledge in general, but also has potentially significant applications in agriculture, forestry, horticulture, and environmental conservation.

Soon after a pollen fertilizes an ovum, the resulting plant zygote begins to specialize, dividing asymmetrically to produce two distinctly different daughter cells. Each of these cells then takes on a specialized role: The larger of the two cells will grow to nourish and support the embryo proper, which develops from the smaller of the two cells. Wolfgang Lukowitz is teasing apart the molecular steps that mediate this specialization event. He has identified a series of three proteins that appear to form part of a signaling pathway, which ensures normal development of the zygote and its daughter cell derivatives. One of the proteins probably associates with a cell surface receptor, another is an enzyme that coordinates biological signaling within cells, and the third protein activates gene expression inside the cell. According to Lukowitz's research, these three proteins form the basis of an important signaling cascade that is likely to be triggered by an as-yet-undiscovered signal emanating from outside the zygote.

Once the plant has begun to send up shoots, form leaves, and eventually flower, a new set of signals must be transmitted as the plant grows. David Jackson studies the transport of regulatory proteins through "plasmodesmata" or channels through which biological signaling molecules can be transported from cell to cell. He is identifying segments of proteins or "motifs" that act as molecular boarding passes, allowing only proteins that bear the motif to be transported through the channel. Jackson and his colleagues have defined the characteristics of the first such motif that enables cell-to-cell protein transport through plasmodesmata. In other work, they have identified genes that control a plant's ability to make branches and regulate the geometries in which its leaves are positioned. Jackson's studies of these "architectural" genes (*abphyl1*, *ramosa3*, and *fasciated ear2*) are revealing fundamental principles of plant growth and form, and may be useful genetic tools for crop improvement.

Marja Timmermans studies how unspecialized stem cells are instructed to take on specialized roles, for example, as parts of the (very different) top layers versus bottom layers of leaves. She and her colleagues are exploring the functions of several gene products (e.g., Leafbladeless1 and Rolled leaf1) in this "dorsoventral axis" stem cell patterning process in maize (corn). Interestingly, Timmermans has discovered that the *Rolled leaf1* gene is regulated through RNA interference (RNAi) by short strands of specialized RNA called microRNAs (miRNAs). miRNAs are emerging as major, previously unrecognized players in a wide variety of organisms and biological processes. Timmermans has shown that particular miRNAs restrict production of the *Rolled leaf1* gene to the top layers of leaves. Her findings reveal that by regulating other genes, miRNAs can act as a developmental signal that ultimately determines the shapes of developing maize leaves. The similarity of these findings to those made through the study of the distantly related plant, *Arabidopsis*, indicates that the control of plant development by RNAi has been highly conserved in evolution.

Part of Rob Martienssen's work involves investigating the role of RNAi in establishing silent chromosome architecture, gene regulation, and plant development. His group has shown that miRNAs restrict production of the *PHABULOSA* transcription factor to the top layers of *Arabidopsis* leaves, where it has a role in leaf polarity. In addition, Martienssen's group directly established a role for RNAi in plant development by showing that mutations in a known component of the RNAi machinery called *Argonaute* yield striking developmental defects, namely, plants with "inside-out" leaves and flowers.

CELL-FATE DECISIONS IN THE EARLY PLANT EMBRYO

W. Lukowitz S. Alabaster
S. Kotkin
J. Williams

In almost all higher plants, the early stages of embryo development follow a predictable sequence of cell divisions and cell-shape changes. This pattern reflects coordinated fate decisions that lay down the reference points of the plant's body plan. Our goal is to identify and understand the genetic network regulating this process. We are working with the small weed *Arabidopsis*, which has proven to be a productive and inexpensive experimental model with good comparability to economically important species.

A MAP Kinase Pathway Promoting Extraembryonic Fate

W. Lukowitz, S. Alabaster

Plant embryo development begins with an asymmetric division of the zygote. This division results in a first and 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.

Our previous work has revealed that the decision between embryonic versus extraembryonic cell fate is regulated by the MAPKK kinase gene *YDA* (see Fig. 1). Loss of *YDA* activity essentially eliminates formation of the extraembryonic suspensor. Conversely, hyperactive forms 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 *YDA* acts as part of a molecular switch: *YDA* is active in the zygote and required for its normal elongation. After the asymmetric division of the zygote, *YDA* activity is turned off in the apical cell and its daughters, which in turn become free to develop the pro-embryo. *YDA* remains active in the basal cell and its daughters to promote formation of the suspensor.

Our genetic screens have identified mutations in two other genes, *SSP* and *GRD*, which suppress suspensor development in a manner similar to loss of *YDA*. This similarity made it seem likely that all three genes operate in the same signaling pathway. A genetic analysis is consistent with this view. Double mutants of *yda* with *ssp* or *grd* do not have a more severe phenotype than *yda* single mutants. Furthermore, dominant 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*.

We have recently succeeded in cloning the *SSP* and *GRD* genes by a map-based approach. *SSP*

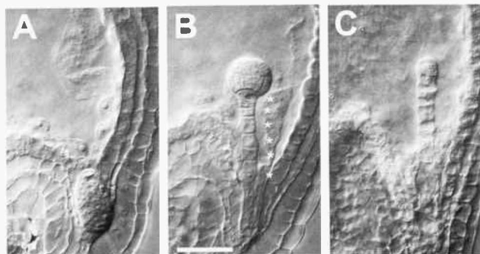

FIGURE 1 The *YDA* MAPKK kinase is part of a molecular switch regulating formation of the extraembryonic suspensor. The suspensor, a file of cells highlighted with stars in *B*, normally supports the developing embryo pushing it toward the center of the immature seed. Loss of *YDA* function results in suspensorless embryos that remain in close contact with the seed coat (*A*). Hyperactive versions of *YDA* suppress development of the embryo, often to the extent that only a stalk of cells is formed (*C*). All panels show an optically cleared immature seed viewed using Nomarski microscopy. Bar, 50 μm.

encodes a cytoplasmic serine-threonine kinase of the Pelle/receptor-like superfamily. This superfamily constitutes by far the largest group of protein kinases in plants (with more than 600 genes in *Arabidopsis*) and contains almost all plant receptor kinases. Plant receptor-like kinases form a monophyletic group with animal kinases of the Pelle family. *Drosophila* Pelle and its mammalian counterparts, the interleukin-1 receptor-associated kinases (IRAKs), interact with cell surface receptors of the Toll/interleukin-1 receptor family, which regulate such diverse processes as embryonic patterning and innate immune recognition. In mammals, activation of IRAK1 triggers activation of transforming growth factor-β-activating kinase 1 (TAK1), which in turn triggers a MAP kinase cascade as well as redistribution of the transcription factor NFκB to the nucleus. By analogy, *SSP* might mediate signal transduction from an as yet unknown cell surface receptor to the *YDA* MAPKK kinase. This idea is supported by the sequence-based prediction that *SSP* is myristoylated at its amino terminus and thus likely peripherally associated with the plasma membrane.

The *GRD* gene encodes an 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 symbiontic 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.

The defining feature of RWP-RK proteins is a conserved sequence stretch around a core of invariant RWP-RK residues (Pfam02042 motif). This sequence contains a leucine zipper motif and shares some similarity with the DNA-binding/dimerization domain of bZIP and bHLH transcription factors, but it does not comply with a strict consensus of either class. Because of this similarity, it has been suggested that RWP-RK proteins function as transcriptional regulators.

Taken together, our results have allowed us to develop a tentative outline of the signaling pathway regulating extraembryonic cell fate in *Arabidopsis*. At the center of the proposed pathway is a MAP kinase cascade that includes the *YDA* gene product and operates downstream from an as yet unknown cell surface receptor. This receptor is most likely triggered by an extracellular stimulus. The receptor-like cytoplasmic kinase *SSP* probably functions in association with or downstream from the receptor complex to activate *YDA*. Targets of the *YDA* MAP kinase cascade likely include transcription factors that execute suspensor-specific developmental programs. A candidate target is the putative transcription factor *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, 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 aerial organs of higher plants are initiated at the tips of shoots by an elaborately organized group of cells termed shoot apical meristem. At the tip of the shoot apical meristem is a small population of stem cells. The size of this stem cell population is thought to be regulated by a feedback loop between the stem cells themselves and the cells directly below them, referred to as 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* expression is limited to

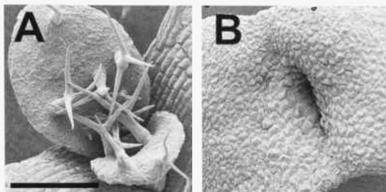

FIGURE 2 Hyperactive version of the MAPKK kinase *YDA* affects development of the apical shoot meristem. Two small leaves have been produced by the shoot apical meristem of a 5-day-old wild-type seedling (*A*). No small leaves are visible in a 5-day-old seedling expressing a hyperactive version of *YDA* (*B*). The cotyledons of this seedling are fused, forming a narrow hollow where the meristem should be located. Both panels show scanning electron micrographs. Bar, 200 µm.

a few cells within the organizing center. These cells, in turn, are thought to provide inductive signals that are required to maintain the stem cells in an undifferentiated state. 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 largely lacking, and no candidate genes have been reported that might function in such a cascade.

We have begun to examine whether the MAPKK kinase *YDA* operates in the CLV signal transduction pathway. The project was triggered by the incidental observation 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. Sean Kotkin, a student in the Partners for the Future program, was able to substantiate this observation by scanning electron microscopy (see Fig. 2). Hyperactive forms of *YDA* frequently cause fusion of the embryonic leaves. The affected seedlings show a narrow hollow at their apex and fail to produce visible organs for many days. Both features are indicative of a defect in the shoot apical meristem. Loss of *YDA* function appears to cause an opposite phenotype, namely, a much enlarged shoot apical meristem.

In collaboration with Venu Reddy in the lab of Elliot Meyerowitz at Caltech, we are developing the tools to forcibly induce *YDA* activity in subsets of cells within the shoot apical meristem. We will follow the effect of these manipulations by imaging cell divisions and marker gene expression in the growing meristems over time. These experiments should allow us to test specific predictions derived from the premise that *YDA* operates in the *CLV* pathway.

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PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen	K. Arney	C. Kidner	D. Roh	M. Tanurdzic
	J.-M. Arroyo	Z. Lippman	M. Ronemus	U. Umamaheswari
	M. Byrne	B. May	R. Shen	M. Vaughn
	D. Garcia	P. Rabinowicz	J. Simorowski	M. Zaratiegui-Biurrun
	D. Goto	U. Ramu	A. Tang	

Epigenetic mechanisms regulate transposon silencing, gene control, and stem cell function in plants, which provide a useful model for higher eukaryotes. Stem cell function and axis formation in *Arabidopsis* depend on *asymmetric leaves1* and *argonaute*, which has an important role in RNA interference. We have demonstrated that spatially restricted small RNA can guide leaf polarity via AGO1. In fission yeast, RNA interference of centromeric transcripts regulate 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, Pablo Rabinowicz, Mary Byrne, and Catherine Kidner left for faculty positions at The Institute for Genome Research (TIGR), the John Innes Institute, and Edinburgh University, respectively, and senior technician Juana-Mari Arroyo returned to Spain. We were joined by postdoctoral fellows Mikel Zaratiegui-Biurrun, Milos Tanurdzic, and Damien Garcia, as well as by our URP, Kat Arney.

Spatially Restricted microRNA Directs Leaf Development via *ARGONAUTE1*

C. Kidner, M. Ronemus, K. Arney, D. Garcia, R. Martienssen

microRNAs (miRNAs) are endogenous small RNA molecules that regulate the expression of matching genes via RNA interference (RNAi). In animals, this regulation occurs by translational control, but in plants, more perfectly matched miRNAs promote site-specific cleavage of the target by argonaute proteins. In *Arabidopsis*, the homeodomain HD-ZIP III genes *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) are expressed on the adaxial (upper) side of leaf primordia where they direct cell fate. They are targets of the miRNAs miR165 and miR166. We have used in situ hybridization to show that miR165 accumulates on the abaxial side of the leaf primordia where it cleaves *PHB* and *PHV* mRNA (Fig. 1). This pattern of expression changes during development, implicating miRNA in signaling between the meristem and the leaf primordium. *argonaute1* (*ago1*) mutants are defective in RNAi and have a pleiotropic phenotype. Leaves and flowers are adaxialized and *PHB* mRNA accumulates throughout the leaf primordium, whereas miR165 is ectopically expressed in the adaxial domain (Fig. 1). Thus, AGO1 regulates both accumulation and function of miRNA, perhaps accounting for enhancement of both loss-of-function and gain-of-function alleles of *PHB*-related genes in *ago1*. We have examined the expression of thousands of genes in normal and mutant lines of *Arabidopsis* using microarrays. Several families of related genes have substantially altered expression in the RNAi mutants, and many of these genes match miRNA. However, not all miRNA targets are altered in abundance, perhaps reflecting the tissue-specific expression of miRNA. Some up-regulated genes may be silenced by second-strand synthesis and degradation in addition to mRNA cleavage. Weak alleles of *ago1* have different phenotypes in different *Arabidopsis* accessions. We are mapping the genetic loci responsible for these variations. The identification of such modifiers will give us new insight into the role of RNAi in natural diversity in plants.

Role of *ASYMMETRIC LEAVES1* in Leaf Polarity and Stem Cell Fate

M. Byrne, D. Garcia, C. Kidner, J.M. Arroyo, R. Martienssen

Hox genes in animals are expressed in discrete domains in early development, responding to a suite of morphogens responsible for axis specification. Gene expression patterns subsequently come under epigenetic control, retaining a memory of early patterning long after the signaling responsible has ceased. In plants, TALE class homeobox genes, such as *SHOOTMERIS*-

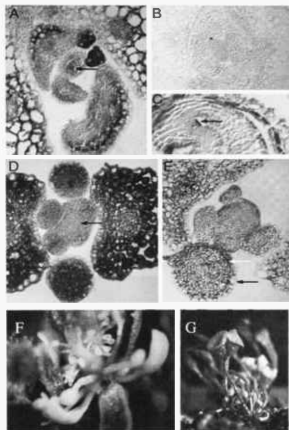

FIGURE 1 miRNA directs leaf polarity via *AGO1*. In *Arabidopsis*, miRNA miR165 corresponding to the homeodomain gene *PHABULOSA* (*PHB*) is restricted to the abaxial side of wild-type leaves (*A*), but it is also found in the meristem in early embryos (*C*). miR165 is more widely distributed in strong *ago1* mutant leaves (*D*). This pattern restricts *PHB* expression in wild type, but not in *ago1* where *PHB* is uniformly expressed (*E*) and directs leaf polarity. The precursor alone (minus the miRNA) cannot be detected in control hybridizations (*B*). The weak allele *ago1-12* (*hairy*) has a leucine residue substituted for an absolutely conserved histidine in the PIWI domain. It has inside-out organs, resulting in ectopic ovules (*F*) and trumpet-shaped leaves (*G*). These phenotypes resemble miRNA-resistant dominant alleles of *PHB*.

TEMLESS, BREVIPEDICELLUS, and *BELLRINGER*, are expressed in the early embryo where they specify the shoot apical meristem, from which postembryonic organs derive. These genes interact in a negative regulatory network with the SANT domain gene *ASYMMETRIC LEAVES1* and the LOB domain gene *ASYMMETRIC LEAVES2* to maintain the distinction between meristem cells and leaf founder cells in postembryonic development. *PHB, PHV*, and *REVOLUTA* belong to another class of homeobox genes, the HD-ZIPIII class, and are expressed on the upper side of the leaf, as well as in meristematic domains. Elsewhere in the plant, RNAi helps to silence these genes via *ARGONAUTE1* and miRNA, as described above. These two systems interact genetically, and we are exploring the mechanism of this interaction via enhancer analysis of *as1*. Loss of adaxial modifiers called *PIGGYBACK* result in ectopic lamina outgrowths on the adaxial leaf surface in *as1*, but have little effect in wild-type backgrounds. Loss of proximodistal modifiers, on the other hand, results in a palmate compound leaf. These modifiers demonstrate a role for AS1 in both proximodistal and dorsoventral axis specification.

Genomic Analysis of RNAi in Fission Yeast

D. Goto, M. Zaratiegui-Biurran, D. Roh, M. Vaughn, R. Martienssen [in collaboration with T. Volpe, NorthWestern University; T. Cech, University of Colorado; J. Bahler, Sanger Center; and G. Thon, University of Copenhagen]

We previously demonstrated that RNAi of centromeric repeat transcripts in fission yeast *S. pombe* was required for chromatin modification and centromere function, and we are extending these studies to the rest of the genome. *ago1*⁻ (argonaute), *dcr1*⁻ (dicer) and *rdr1*⁻ (RNA-dependent RNA polymerase) mutants have very similar microarray profiles: Few genes are up-regulated, suggesting a limited role for miRNA, but centromeric small interfering RNA (siRNA) matches a *recQ* homolog found at the telomeres. Silencing of this gene depends on Ago1 and Dcr1, but not on Rdr1, and is presumably posttranscriptional. Transcripts from retrotransposable elements are only slightly up-regulated in RNAi mutants despite the loss of H3mK9 from their long terminal repeats (LTRs). Microarray profiles of RNAi mutants were similar to those from the H3K9 methyltransferase *clr4*⁻, but profiles from other silencing mutants had more widespread effects. These results are in agreement with our observations in *Arabidopsis* that transposon silencing can be maintained independently of RNAi. We are currently using tiling arrays of fission yeast heterochromatin to more precisely map centromeric transcripts as well as chromatin modifications.

Several genes involved in RNAi and heterochromatic silencing are found in the genome of fission yeast but not in baker's yeast *Saccharomyces cerevisiae*, suggesting that other RNAi genes may be coeliminated. We used phyletic profiling of five higher eukaryote genomes, the two yeast species, and several other fungal species to predict likely *Schizosaccharomyces pombe* participants in RNAi-mediated silencing. We refined this candidate list by using protein interaction data from higher eukaryotes to identify *S. pombe* proteins, many of which were hypothetical proteins, that might interact with the RNAi machinery. These and other analyses have been used to construct an enhanced *S. pombe* model organism database.

Transposable Elements, Epigenetic Control, and the Origin of Heterochromatin

Z. Lippman, M. Vaughn, B. May, R. Martienssen [in collaboration with W.R. McCombie and V. Mittal, Cold Spring Harbor Laboratory; J. Carrington, Oregon State University; R.W. Doerge, Purdue University; and V. Colot, INRA/CNRS Evry, France]

Transposable elements and related tandem repeats are large components of eukaryotic genomes and are enriched in heterochromatic regions. The transcriptional activity of these sequences is suppressed by epigenetic silencing mechanisms that involve the covalent modification of DNA and associated histones. In particular, methylation of cytosine residues in DNA and of histone H3 Lys-9 are evolutionarily conserved modifications involved in heterochromatin silencing. In the fission yeast *S. pombe*, which lacks DNA methylation, histone H3 Lys-9 methylation and silencing of centromeric repeats are regulated by RNAi. In plant genomes, DNA methylation is primarily restricted to transposons and related repeats. In an effort to determine if RNAi, Lys-9 methylation, and DNA methylation are mechanistically linked in repeat sequence silencing, epigenetic mutants in *Arabidopsis thaliana* affecting each process were assayed for silencing defects and compared to wild-type plants as well as to each other. Transposons and tandem repeats, but not genes, were selectively enriched in both DNA methylation and histone H3 Lys-9 methylation, which correlated with the presence of siRNA. Despite their similar localization in cytologically defined heterochromatin, different classes of transposons were subjected to different forms of epigenetic regulation, suggesting the existence of multiple silencing pathways or complexes. The chromatin remodeling gene *Decrease in DNA Methylation1* (*DDM1*), the maintenance CG methyltransferase (*MET1*), and the type I histone deacetylase *Silencing 1* (*SIL1*) are required to maintain most transposon silencing, and *DDM1* and *MET1* further influence the accumulation of siRNA. Reestablishment of silencing occurred when *met1* and *sil1* were backcrossed to wild type, but only when siRNA was retained in the mutants, suggesting a role for siRNA for silencing in *cis*. Genes with transposons inserted within or near them were under transposon control and, consequently, regulated by *DDM1*, *MET1*, and RNAi. For example, transposons and tandem repeats integrated near the imprinted genes *FWA* and *MEDEA* correspond to siRNA and contribute to silencing, although their role in imprinting is still unclear.

Arabidopsis Centromeric Repeat Transcripts

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

Centromeres interact with the spindle apparatus to enable segregation of chromosomes into daughter cells and are typically flanked by many thousands of satellite repeats interspersed with retrotransposons. Although the function of these repeats is unclear, centromere selection has a strong epigenetic component. Yeast centromeres are much simpler, but those of *S. pombe* are also flanked by heterochromatic repeats whose transcription contributes to centromere function via RNAi. We have investigated the transcription and modification of centromeric repeats in *A. thaliana*, which unlike yeast has methylated DNA. One strand of the 180-bp satellite repeat arrays is transcribed, and the transcripts remain associated with heterochromatin. These transcripts are posttranscriptionally silenced by RNAi and histone modification, but not by the canonical CpG DNA methyltransferase *MET1*. Other 180-bp repeats are transcribed from both strands and silenced by *MET1*, *SIL1*, and *DDM1*. Centromeric transcripts of the first class are inherited epigenetically. Those of the second class are also inherited epigenetically from *ddm1*, which loses siRNA, but not from *met1* and *sil1*, which retain siRNA. Histone H3 Lys-9 methylation depends on *AGO1* as well as on the histone modification machinery. Cytological examination has yet to reveal the presence of lagging chromosomes at anaphase in any of these mutants, although some lose a comparable proportion of histone H3 Lys-9 methylation to fission yeast mutants that display chromosome segregation defects. It is possible that differences in plant and yeast cell division mechanisms are responsible.

Chromatin Modification, DNA Replication, and Polyploidy

M. Tanurdzic, Z. Lippman, P. Rabinowicz, M. Vaughn, R. Martienssen [in collaboration with L. Comai, University of Washington; T. Osborn, University of Wisconsin; W. Thompson, North Carolina State University; J. Murray, Cambridge University; and R.W. Doerge, Purdue University]

Polyploidy in plants results in a variety of genetic and epigenetic changes in gene expression from generation to generation following the establishment of allopoly-

ploids. 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*. We have found that changes in heterochromatin are relatively modest, but that some genes and some transposons are activated in the allotetraploids. We are also comparing diploid chromatin modification profiles to the pattern of replication of chromosomal DNA. By labeling synchronized cells with BrdU, and immunoprecipitating the replicated DNA at different time points, we can determine the approximate location of chromosomal origins in these cells. We will compare these profiles to gene expression, DNA methylation, and chromatin modification profiles.

Development of Informatics Resources for the *Arabidopsis* Genome

M. Vaughn, R. Martienssen

We have constructed a model organism database system for *A. thaliana* based on PostgreSQL, Apache, and Perl. This resource integrates gene information derived from the reference TIGR genome releases; third party annotations provided by TAIR, MIPS, and others; and notes derived from published and in-house analyses. Annotations can be queried by a simple Web form or, for more expressivity, via a Perl interface which implements BioPerl objects. This system is very flexible and has been extended to treat features from our genomic tiling arrays, the Affymetrix AtGenome1 and ATH1 arrays, the TAMU 26k 60-mer array, and the Salk Institute whole-genome array as computational features mapped onto the genome. Values are assigned to the array features based on normalized hybridization values from any number of experiments, facilitating comparison of equivalent features across microarray platforms and experiments. Other computations, such as copy number, small RNA homology, MPSS tag abundance, and insertional mutant flanking sequence locations also extend the default genome annotation. We have taken full advantage of the open-source Generic Genome Browser platform (from L. Stein, Cold Spring Harbor Laboratory) to construct a Web-based visualization tool to present the data contained in the annotation and microarray database as an easy-to-interpret, track-based image (http://chromatin.cshl.org/ddm1/). The integrated visualization of annotation, computation, and profiling data has allowed scientists from Cold Spring Harbor Laboratory, INRA/CNRS Plant Genomics Research Unit (Evry, France), and the NSF Functional Genomics of Polyploidy project to explore questions of epigenomic context.

Methylation Filtration of Sorghum and Other Plant Genomes

P. Rabinowicz, R. Martienssen [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory, and Orion Genomics LLC, St Louis, Missouri]

Methylation filtration (MF) is a gene-enrichment technology that has been successfully applied to sorghum and maize, where gene enrichment is proportional to genome size. We have applied MF to a broad variety of plant species spanning a wide range of genome sizes. Enrichment for genes was observed in all species tested, from nonvascular to vascular plants, but differences between the expected and the observed level of enrichment were noted. In wheat and pine, only a low level of gene enrichment was observed, which may reflect an abundance of methylated pseudogenes in these recent polyploids. *Sorghum bicolor* is a close relative of maize and is a staple crop in Africa and much of the developing world because of its superior tolerance of arid growth conditions. We have generated sequence from the hypomethylated portion of the sorghum genome. At least 90% of the genes have been tagged with an average coverage across more than 60% of their length after generating a raw coverage of less than 300 Mb of the 735-Mb genome. Methylation filtration preferentially captures exons and introns, promoters, miRNAs, and simple sequence repeats and minimizes interspersed repeats, thus providing a robust view of the functional parts of the genome. Thousands of hypothetical gene predictions in rice and *Arabidopsis* are supported by the sorghum set.

Functional Genomics with Transposons in Maize and *Arabidopsis*

B. May, M. Vaughn, D. Roh, J. Simorowski, J.-M. Arroyo, R. Shen, A. Tang, U. Ramu, R. Martienssen [in collaboration with W.R. McCombie, L. Stein, and D. Spector, Cold Spring Harbor Laboratory, and E. Lam, Rutgers University]

Our collection of *Arabidopsis* gene-trap and enhancer trap transposon lines has grown to more than 37,700 individual insertions, about half of which have been

mapped to the genome by polymerase chain reaction (PCR) and sequencing. The resulting knockouts, along with phenotypic and expression data, are made available to the public via TRAPPER (http://genetrap.cshl.org), an interactive database and ordering system. We have expanded this system to include gene-trap transposons with *lacI*-GFP chromosome charting beacons that enable chromosomal locations to be visualized in the nucleus. We continue to serve the maize community through MTM (maize-targeted mutagenesis), an efficient system for site-selected transposon mutagenesis. MTM selects insertions in genes of interest from a library of 45,000 plants using PCR. Pedigree, knockout, sequence, phenotype, and other information are stored in a powerful interactive database (mtmDB) that enables analysis of the entire population as well as handling knockout requests. By monitoring *Mutator* activity, we conclude that more than one half of all mutations arising in this population are suppressed on losing *Mutator* activity. More than 200 orders were filled for maize and *Arabidopsis* lines in 2004.

Arabidopsis BRCA2

J. Simorowski, U. Ramu, R. Martienssen

The breast cancer susceptibility 2 gene *BRCA2* has been studied extensively and has been found in mammals, birds, plants, and fungi. Mutations in the human ortholog predispose the patient to breast and ovarian tumors. The model plant *A. thaliana* has two copies of *BRCA2* in its genome, and we have been studying the phenotypes corresponding to insertional mutagenesis of these genes. Using our collection of gene-trap lines, as well as mutant lines from other collections, we have created *BRCA2* double mutants. Although the single homozygous *BRCA2* plants are phenotypically normal, the double homozygous plants have a marked reduction in fertility, ranging from semisterile to fully sterile. Double heterozygous mutant plants are phenotypically similar to wild-type and single mutant plants. We are investigating triple mutants between the *BRCA2* lines and *BRCA2*-interacting genes, such as *RAD51*, which encodes a protein involved in DNA repair that interacts directly with *BRCA2* during recombination.

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PLANT CELL SIGNALING AND GENETICS

D. Jackson Y. Benitez K. Lau N. Satoh-Nagasawa
P. Bommert B.H. Lee J. Wang
M. Cilia J. Linder P. Yin
M. Jan A. Mohanty

Our research aims to understand fundamental mechanisms in developmental biology, and how morphology and architecture arise and are maintained in biological systems. We use plants as a convenient model system because of their ease of genetic manipulation, interesting developmental strategies, and applications to agriculture and our food supply. In the past year, we have concentrated on the isolation of novel genes that regulate development and have invested efforts to characterize the function of recently sequenced plant genomes. In an era where we can now predict the identity of all genes required to make a plant, such technologies are increasingly important. They will allow us to understand the "big picture" of interacting proteins and pathways that create the integrated organism.

We also continue to advance our understanding of signaling pathways that regulate stem cell fate and development. We have found novel mutants in cell-to-cell protein trafficking and are pursuing their isolation. We also identified genetic interactions regulating stem cell proliferation in the maize inflorescence and isolated a novel phosphatase gene that controls inflorescence branching. As plant development is also intimately associated with hormonal signaling, we are investigating a novel role for cytokinin hormones in stem cell proliferation and leaf patterning.

Isolation of the *RAMOSA3* Gene of Maize by Positional Cloning

N. Satoh-Nagasawa, J. Linder [in collaboration with N. Nagasawa and H. Sakai, DuPont Agriculture and Nutrition, Wilmington, Delaware]

Inflorescence branching is a major yield factor in many crops and is determined by the activities of shoot meristems. Because of our general interest in inflorescence development and shoot meristem organization, and the importance of inflorescence branching in development of maize as a crop, we have been continuing the characterization of the *ramosa3* (*ra3*) mutant, which has enhanced inflorescence branching. We observed *ra3* ear development by scanning electron microscopy and found that, in general, *RA3* functions to impose determinacy and identity on the different meristem types in the inflorescences. Although the inflorescence meristems are normal, the spikelet pair, spikelet, and floral meristems are all more indeterminate and have converted or delayed identity in *ra3* mutants compared to wild type.

Double-mutant analyses suggest that *RA3* acts synergistically with another branching gene, *RA2*, because the double mutants are much more severe than either single mutant. To more fully understand the role of *RA3* in inflorescence development, we used the emerging genomic resources in maize to isolate the gene by positional cloning. *RA3* encodes a protein with a highly conserved phosphatase domain, suggesting that it acts in a signal transduction cascade to regulate meristem determinacy and identity. This class of phosphatases has been implicated in stress protection and in metabolic signaling, and our discovery is the first indication that these proteins are also involved in developmental regulation.

Reverse transcriptase–polymerase chain reaction (RT-PCR) and in situ hybridization experiments indicate that *RA3* is expressed in the developing inflorescences, in a cup-shaped domain at the base of the meristems (Fig. 1A). This expression pattern allows us to hypothesize that *RA3* may process a signal that moves into the meristem to regulate determinacy and identity. *RA3* also has an interesting genomic organization; sequencing of a *RA3* BAC clone indicates that the adjacent gene is a *RA3* homolog, which we called *sister of RA3* (*SRA*). However, there is only a single ortholog of *RA3* in rice, which leads us to imagine an interesting evolutionary story whereby *RA3* may have evolved a developmental role relatively recently. Since there are limited studies at the interface between metabolic control and development, analysis of *RA3* may give unique insights into the mechanisms of branching in maize inflorescences as well as in other crop plants.

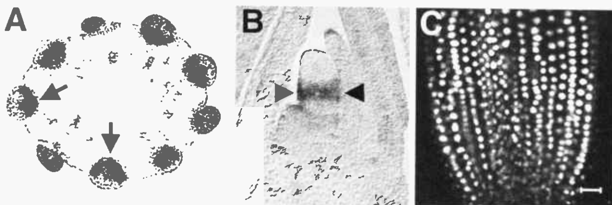

FIGURE 1 Patterns in plant development. (*A*) The *RAMOSA3* gene is expressed in cup-shaped domains (*arrows*) at the base of meristems and may process a signal that regulates their determinacy. (*B*) ABPHYL1 is expressed in stripes in the shoot apical meristem (*arrowheads*). (*C*) Subcellular localization of a YFP-tagged *Arabidopsis* protein of unknown function shows that it localizes to the nuclei of root tips. Bar, 20 μm.

Studies of Maize Phyllotaxy

B.H. Lee, J. Wang

Mutations in the cytokinin-inducible response regulator homolog *ABPHYL1* (*ABPH1*) in maize result in decussate phyllotaxy, where leaves are initiated in opposite pairs. This is very distinct from the normal pattern, where leaves are initiated singly in an alternating pattern. To gain insight into the functions of this gene family in regulating leaf patterns across different plant species, we are now using the model dicot *Arabidopsis*. Mutants defective in *Arabidopsis* cytokinin-inducible response regulators and other cytokinin signaling components have been obtained, and their developmental phenotypes are being monitored.

Auxin is another classical plant hormone that has long been coupled with cytokinins in its mechanism of action, and it has also been implicated in the regulation of phyllotaxy. To examine the interaction between auxin and cytokinin in the regulation of phyllotaxy, two approaches are being used. First, we are analyzing the *ABPH1* expression domain in auxin polar transport mutants or auxin polar transport inhibitor (*N*-1-naphthylphthalamic acid [NPA])-treated plants. *ABPH1* is expressed in stripes in the meristem that predict the position of leaf initiation (Fig. 1B). Our preliminary in situ hybridization data suggest that NPA treatment alters the *ABPH1* expression domain in the meristem. An *ABPH1* antibody has been made and will be also used for this expression domain analysis. Second, using an anti-PINFORMED1 antibody, we will also investigate the expression patterns of putative polar auxin transport proteins in *abph1* mutants. To facilitate the studies of the *ABPH1* expression domain, an *ABPH1* yellow fluorescent protein (YFP) transgenic maize line is being generated. The YFP has been internally integrated in-frame into the *ABPH1*-coding sequence under the control of its own promoter, using the fluorescent tagging of full-length proteins (FTFLP) method developed in our lab.

To identify additional factors involved in the control of phyllotaxy, we are characterizing another maize phyllotaxy mutant, *abph2*. The *abph2* mutant shoot meristem size appears to be bigger than that of the wild type, and the *ABPH1* expression domain is enlarged. These results suggest that *ABPH2* might function in the same phyllotaxy regulatory pathway as *ABPH1*. We are planning to map *ABPH2*, with the goal of isolating the gene by positional cloning.

Toward Characterization of the *Arabidopsis*, Maize, and Rice Proteomes by Native Expression of Tagged Full-length Proteins

A. Mohanty [in collaboration with M. Myers and W.R. McCombie, Cold Spring Harbor Laboratory]

Arabidopsis thaliana, the first plant to have its genome completely sequenced, has more than 29,000 predicted genes. However, to this date, less than 10% of the gene activities have been verified experimentally, and there are only very limited reports of systematic analysis of gene function. To address this problem, we have developed high-throughput methodologies to tag genes for functional analysis, including subcellular localization and identification of interacting proteins. Our first use of this approach, termed FTFLP, aims to analyze expression patterns and subcellular localization of *Arabidopsis* gene products *in planta*. Our FTFLP-based approach offers two significant advantages: (1) It produces internally tagged full-length proteins that are likely to exhibit their correct intracellular localization, and (2) it yields information about the tissue specificity of gene expression, by the use of native pro-

moters. To demonstrate how FTFLP may be used for characterization of the *Arabidopsis* proteome, we tagged a series of known proteins with diverse subcellular targeting patterns, as well as several proteins with unknown function, and unassigned subcellular localization (Fig. 1C). The results are available through a Web database (http://aztec.stanford.edu/gfp/).

Recently, we have applied this methodology to the economically important crops rice and maize. Rice is the first cereal crop plant to have its genome completely sequenced. However, the function of only a handful of rice genes is known. For the rice-tagging project, we are using a plant-optimized tandem affinity purification (TAP) tag with the aim to study protein-protein interactions. We are currently generating TAP-tagged lines for purification of the protein complexes, with the aim of making a comprehensive protein interaction map. We are also applying the fluorescent tagging approach to maize, to generate fluorescent-protein-tagged lines for developmental and genomics studies. It is expected that our study will pave the way for deciphering the function of a large number of plant genes at the cellular level.

Molecular Genetic Characterization of Plant Intercellular Channels

Y. Benitez, M. Cilia, M. Jan, J. Wang, P. Yin (in collaboration with J.Y. Kim, Gyeongsang National University, Korea)

The intercellular transport of metabolites, the spread of RNAi (RNA interference), and viral movement are all processes regulated by the permeability of plasmodesmata (PD) channels. PDs are also involved in regulating symplastic communication during development and plant cell fate. Despite their importance, we understand very little about the mechanisms regulating either targeted or nontargeted movement through PD. Our group has invested significant effort in screens to isolate genes involved in PD transport pathways and in the identification of signals for PD targeting.

In the last year, we reported the isolation of a number of mutants arrested in the intercellular movement of the green fluorescent protein (GFP) named *gfp altered trafficking* (*gat*, pronounced "gate"). We have characterized two of these so far; *gat1*, for which we have two alleles, is recessive and has severe developmental phenotypes. In contrast, *gat3* is dominant and morphologically normal. The *gat* mutants were isolated based on their block in GFP transport out of the conductive phloem tissues, but they also differ in GFP movement, and perhaps in PD size exclusion limit, in nonphloem tissues. For instance, *gat1* is also impaired in GFP movement between epidermal cells compared with wild-type seedlings. However, GFP moves normally between *gat3* epidermal cells. We also observed PDs indirectly using the marker PD41, a GFP partial cDNA fusion that labels PDs in wild-type seedlings. *gat1* mutant seedlings showed only diffuse PD41 GFP fluorescence at the cell periphery, suggesting that PDs are disrupted in this mutant. *gat1* mutants make only one or two small leaf primordia and have reduced expression of a meristem regulatory gene (*STM*).

We are now fine mapping two of the *gat* mutants, in order to identify which genes are disrupted. We have mapped *gat1* to a small region of chromosome 2 around the marker THY1, and *gat2*, which has a phenotype similar to that of *gat1*, to chromosome 1. In the meantime, we continue in-depth analysis of the *gat* mutants. We will assess movement of RNAi signals or of low-molecular-weight dyes and observe PD structure in the mutants by transmission electron microscopy or using fluorescent markers to understand the roles of the *GAT* genes in cell-to-cell transport.

We also designed a new approach to ask what proportion of *Arabidopsis* proteins are capable of targeted trafficking through PD, to identify additional PD-trafficking signals, and to isolate mutants in targeted trafficking of KNOTTED1 (KN1). KN1 was the first plant developmental protein found to traffic through PD, and it is now the founding member of a growing class of noncell autonomous developmental proteins. For this aim, we will use a novel system that we developed to easily monitor protein trafficking by phenotypic screening of leaf hairs or trichomes (Fig. 2A,B). We hope that this approach will provide genome-wide insight into the prevalence of targeted PD trafficking and identify short trafficking signals that could be useful in targeting predictions or to regulate the trafficking of other proteins.

Regulation of Meristem Size and Seed Yield in Maize

P. Bommert, K. Lau, J. Wang

Plant growth is characterized by reiterative developmental events that depend on the activity of meristems. The aerial part of the plant body is elaborated from the shoot apical meristem (SAM), which produces leaves, stems, and axillary meristems in a repetitive manner. Established during embryogenesis, the SAM serves as a reservoir of stem cells during the plant life cycle. It

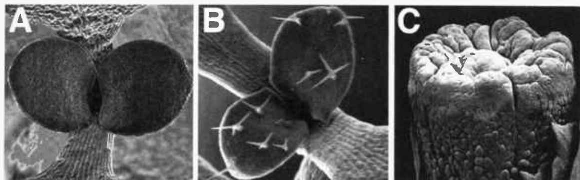

FIGURE 2 Morphology and plant development. The *glabrous1* mutant of *Arabidopsis* lacks leaf hairs or trichomes (*A*). We devised a scheme to complement this mutation using cell-to-cell trafficking of the GL1 protein fused to KN1, which results in trichome production (*B*). In *C*, a greatly enlarged maize ear meristem is produced by a combination of mutations in *td1* and *fea2* genes. These genes may regulate yield through controlling seed number on the ear.

can be divided morphologically and functionally into a central zone (CZ), in which the stem cells are located, and a surrounding peripheral zone (PZ), where cells become incorporated into lateral organ primordia.

In *Arabidopsis*, analysis of fasciated mutants has identified some of the genes involved in maintaining the balance between stem cell fate in the CZ and the onset of differentiation in the PZ. Mutations in genes in the CLAVATA signaling pathway lead to increased cell numbers in meristems. The current working model postulates that the CLAVATA3 ligand is secreted from stem cells and binds to a receptor complex containing CLAVATA1, CLAVATA2, and other signal transduction molecules. Activation of the receptor results in the restriction of transcription of *WUSCHEL*, a positive regulator of stem cell identity.

We are investigating the role of the *thick tassel dwarf1* (*td1*) and *fasciated ear2* (*fea2*) genes in maize, which encode CLAVATA1 and 2 orthologs. Both mutants develop severely enlarged inflorescence meristems, indicating that they function in a manner similar to *CLV1* and *2* in *Arabidopsis*. The phenotypic analysis of *td1, fea2* double mutants, however, demonstrated that *td1* and *fea2* do not act exclusively in a receptor complex to regulate meristem size (Fig. 2C). To gain insight into the nature of the receptor complex, we plan to isolate the *fea2* complex by using a transgenic FEA2 TAP-tagged maize line. A construct containing the FEA2-coding sequence fused to a plant-optimized TAP tag has been generated and is currently being transformed into maize.

Besides this biochemical approach, we also address the question of whether *fea2* has a role in the evolution of maize, especially with respect to the cob structure of maize, which is unique among the grasses. In teosinte, the ancestor of maize, the seeds are arranged in two alternating rows, whereas modern maize lines have up to 18 rows of seeds. Seed row number is dependent on the number of meristems, called spikelet pair meristems (SPMs), initiated by the inflorescence meristem. It is tempting to speculate that the number of SPMs is directly dependent on the size of the inflorescence meristem. Our analysis of inflorescence meristem size and seed row number in different maize inbred lines supports this assumption, as we observe a significant positive correlation. To address whether allelic variation in the *fea2* gene also has an impact on seed row number, we are performing a high-resolution QTL analysis using a maize recombinant inbred population. Our analysis of several independent data sets indicates that indeed, allelic variation in *fea2* may regulate seed row number, as one of the most significant QTLs is located on chromosome 4, corresponding to the *fea2* map position.

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PLANT DEVELOPMENTAL GENETICS

M. Timmermans M. Guo, M. Juarez, S. Madi, P. Mazur, F. Nogueira, T. Phelps-Durr, J. Thomas

ANALYSES OF GENES INVOLVED IN MERISTEM FUNCTION AND LEAF INITIATION

This work was done in collaboration with M. Scanlon, University of Georgia, Athens; P. Schnable, Iowa State University, Ames; B. Buckner and D. Janick-Buckner, Truman State University Kirksvile, Missouri.

One of the most intriguing aspects of plants is their ability to generate organs postembryonically. 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. These different cell types are organized in 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 (Fig. 1). In addition, meristems comprise distinct epidermal and subepidermal cell layers. Traditional genetic analyses have identified several genes that are required for meristem function or for the development of lateral organs from the SAM. Such studies have also shown the importance of this meristematic organization for normal plant development.

To identify novel and potentially redundant or essential genes that function in discrete domains of the SAM or developing leaf primordia, we are using a technique called laser capture microdissection (LCM). LCM 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. During the past year, we have 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. We have found that acetone-fixed and paraffin-embedded SAMs preserve tissue integrity without compromising the efficiency of LCM and subsequent RNA isolation. RNA collected from as few as a thousand cells was 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, for instance, to *Arabidopsis*. However, maize has the disadvantage that its genome project is still at an early stage. To identify genes that should be represented on the microarrays, we have also initiated a "small" expressed sequence tag (EST) project. We have currently sequenced nearly 10,000 ESTs from meristem-enriched cDNA libraries, and the ultimate goal is to generate approximately 35,000 3′ ESTs. We intend to compare global expression patterns between approximately 20 different functional meristematic and leaf primordia domains, or experimental conditions. Examples of the types of comparisons are as follows: (1) between the expression profiles of the stem

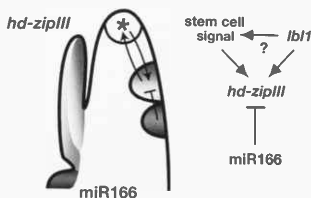

FIGURE 1 Genetic interactions between stem cells and their differentiating descendants in the incipient leaf. Adaxial/abaxial patterning of lateral organs requires two meristem-derived signals. One signal, which may exert its effects via the START lipid-sterol-binding domain of HD-ZIPIII proteins, is derived from the stem cells in the SAM (*). The miR166 signal initially accumulates immediately below the incipient leaf but gradually spreads into the abaxial domain, thus restricting *hd-zipIII* expression to the adaxial side. *hd-zipIII* expression also depends on *lbl1*, which may act via the stem-cell-derived signal. *hd-zipIII* genes specify adaxial fate and signal back to the SAM to maintain stem cell function.

cells, their immediate derivatives in the SAM, and the initials of lateral organs; (2) between the different tissue layers of the SAM; (3) between different stages of leaf development; (4) between 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 will be verified by more traditional expression analyses such as real-time reverse transcriptase–polymerase chain reaction (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.

ADAXIAL/ABAXIAL PATTERNING OF LATERAL ORGANS IN MAIZE

Signaling between the different cell populations within the meristem is important for stem cell function. In addition, stem cell function and meristem maintenance rely on signals from differentiating cells within developing organs. A positive feedback loop exists between stem cells in the SAM and their differentiating derivatives in the leaf. Early studies indicated that a stem-cell-derived signal sets up adaxial/abaxial polarity in lateral organs, which leads to distinct upper and lower leaf surfaces. This past year, we have shown that adaxial/abaxial patterning also requires a microRNA (miRNA) signal that originates within the meristem (Fig. 1). Both signals act on members of the class III *homeodomain leucine zipper* (*hd-zipIII*) family, which specify upper cell fate but are also required for SAM formation and maintenance.

The semidominant *Rolled leaf1* (*Rld1*) mutant from maize is characterized by an upward curling of the leaf blade due to adaxialization or partial reversal of leaf polarity. We have cloned the *rld1* gene and found that it encodes a member of the HD-ZIP III family of proteins. *rld1* is normally expressed in the central zone of the SAM, in vasculature and, consistent with a role for *rld1* in adaxial cell fate specification, in nondetermined cells on the adaxial side of leaf primordia. The meristematic expression pattern of *rld1* was unaffected in *Rld1*, but we observed that *rld1* is misexpressed on the abaxial side of *Rld1* mutant leaves. These and other observations indicate that polarized *hd-zipIII* expression is essential for the formation of distinct upper and lower leaf surfaces. However, our in situ hybridization analysis showed that *rld1* and other *hd-zipIII* family members are expressed uniformly throughout the leaf founder cells. We were able to show that polarized *hd-zipIII* expression is subsequently set up by an miRNA-directed RNA-interference-like process.

Transcripts of the *hd-zipIII* genes contain a complementary site for miRNA165 (miR165) and 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. We found that all four dominant *Rld1* alleles result from a single nucleotide change in this site. This, together with the misexpression of *rld1* in the *Rld1* mutant, suggests that miRNAs mediate the cleavage of *rld1* transcripts on the abaxial side of leaf primordia. In other words, miR165/166 may specify abaxial cell fate by restricting expression of *rld1* and other *hd-zipIII* genes to the adaxial side. To test directly whether miRNAs can establish patterns of tissue differentiation during development, we used in situ hybridization to determine a precise cellular expression pattern for miR166. We found that *rld1* and miR166 exhibit complementary expression patterns in developing leaf primordia consistent with a role for miR166 in the spatial regulation of *rld1* transcripts. More importantly, our in situ hybridization data indicated that the peak of miR166 expression occurs just below the domain in which miR166 acts on the *hd-zipIII* genes and that an expression gradient of miR166 is established in developing leaves. These observations led us to suggest that miR166 is mobile, and this offers the intriguing possibility that miRNAs can function as morphogen-type signals in development. We are currently studying the basis for this dynamic expression pattern of miR166, by determining the *cis*- and *trans*-acting factors required for miR166 expression and possible movement.

As mentioned above, adaxial/abaxial patterning requires a second meristem-derived signal. This stem cell signal is essential for adaxial cell fate and is thought to act via the HD-ZIPIII proteins, which contain a START-like lipid-sterol-binding domain. As a first step toward identifying the potential adaxializing signal, we have purified the START domain from RLD1 and are using biochemical approaches to determine which lipid-sterols bind to this START domain. In addition, we are taking a genetic approach to identify genes that produce, convey, or respond to the stem cell signal. Recessive mutations in *leafbladeless1*

(*lbl1*) cause the formation of abaxialized leaves, suggesting that *lbl1* is required for adaxial cell identity. Expression of the *hd-zipIII* genes in the SAM and on the adaxial side of leaf primordia is reduced in *lbl1*, indicating that *lbl1* acts upstream of *rld1* and other *hd-zipIII* family members. Interestingly, the level and pattern of *rld1* expression in the vasculature of *lbl1* are not affected. Because vascular *hd-zipIII* expression is also controlled by miR166, these observations suggest that *lbl1* controls *hd-zipIII* expression independent of miR166. We are in the process of cloning the *lbl1* gene using transposon-tagging and map-based approaches.

In the past few years, genetic analyses in several diverse plant species have shown that a signal for the adaxial side of the leaf is required to maintain the SAM and its stem cell population. We have isolated several mutant alleles of *lbl1*. The most severe alleles fail to initiate a SAM during embryogenesis. Mutant embryos lack *hd-zipIII* expression. In addition, these embryos fail to express *knotted1*, which encodes a homeodomain protein known to be required for meristem function in a wide variety of plant species. By studying the genetic pathway involving *lbl1, rld1*, and miR166, we hope to improve our understanding of the interdependence of stem cells and their differentiating descendants and the role of miRNAs as developmental signals.

REPRESSION OF STEM CELL FATE DURING ORGAN DEVELOPMENT

This work was carried out with contributions from Arooba Alam and Samantha Meyerhoz, Locust Valley High School.

knotted1 and other members of the *knox* homeobox gene family are required for meristem initiation and maintenance. These genes are expressed in the indeterminate cells of the SAM but are excluded from determinate leaf founder cells. Moreover, down-regulation of *knox* genes is essential for normal leaf development. Misexpression of *knox* genes within developing leaves leads to cell division and differentiation defects, not unlike tumor formation in animals. The *rough sheath2* (*rs2*) gene from maize and the *ASYMMETRIC LEAVES1* (*AS1*) gene from *Arabidopsis* encode orthologous MYB-domain proteins that act as negative regulators of *knox* gene expression. On the basis of expression and genetic analyses, we proposed that these proteins act as epigenetic regulators to keep *knox* genes in an "off" state in developing lateral organs, thus preventing differentiating cells from reverting into indeterminate stem cells. Our recent observations confirm this hypothesis. We have shown that RS2 and AS1 form highly conserved repressor complexes that include the zinc finger transcription factor ASYMMETRIC LEAVES2 (AS2), an RNA-binding protein named RIK, and the histone chaperone HIRA and may recruit protein phosphatase 2A to the *knox* loci. HIRA proteins are histone chaperones that have a role in both euchromatic and heterochromatic gene silencing in yeast and animals. The mechanism by which HIRA establishes euchromatic gene silencing is unknown. HIRA is a unique histone chaperone because it can incorporate or exchange nucleosomes and histone variants independent of DNA synthesis. In addition, HIRA can interact with histone deacetylases. Both 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.

To establish that the AS1 complex binds directly to the knox loci, we have generated transgenic lines that express a fusion protein comprising the DNA-binding domain of the floral transcription factor LEAFY (LFY-DB) and the non-MYB domain of AS1. More than half of the plants carrying this transgene failed to form flowers, which is typical of strong *lfy* mutants. Control plants expressing the LFY-DB alone were normal, suggesting that LFY-DB expression does not cause a dominant-negative phenotype. Replacement of the LFY activation domain with the non-MYB domain of AS1 thus blocks activation of LFY targets. This suggests that AS1 acts as a transcriptional corepressor and may bind directly to the *knox* genes. We therefore used chromatin immunoprecipitation to determine which regions of *BP*, one of the *knox* genes, mediate AS1 complex binding. We have identified three regions within the *BP* gene that interact with the AS1 complex. We are currently defining these *cis*-acting sequences more precisely and are analyzing which proteins in the AS1 complex mediate these interactions.

We are also using the reverse genetic resources available in *Arabidopsis* to determine the roles of the AS1-interacting proteins in plant development, particularly in *knox* gene regulation. Loss-of-function mutations in AS2 are known to affect *knox* gene silencing during leaf development. We have isolated mutations in the *Arabidopsis* RIK and HIRA proteins. Loss-of-function mutants in RIK are indistinguishable from wild type. In contrast, weak mutant alleles of *hira* develop leaf and floral defects that resemble the defects observed in the *as1* and *as2* mutants. Moreover, *hira* mutant leaves misexpress several *knox* genes. AS2 expression is unaffected in *hira* mutants and *AS1* tran-

script levels are increased, indicating that the defects in *hira* do not result from loss of *AS1* or *AS2*. Double-mutant analysis revealed that *hira* enhances the *as1* and *as2* mutant defects. Taken together, our observations suggest AS1 and AS2 assemble into a protein complex that targets HIRA and possibly other chromatin remodeling proteins to the *knox* loci in organ founder cells. As a result, stem cell fate remains repressed in determinate differentiating cells of developing lateral organs. Deletion of HIRA in humans contributes to the DiGeorge syndrome, which is thought to arise from developmental defects in the neural crest. Thus, HIRA may also have a role in the repression of stem cell fate in animals. Our studies could therefore highlight fundamental properties of establishing/maintaining determinacy in plants and animals.

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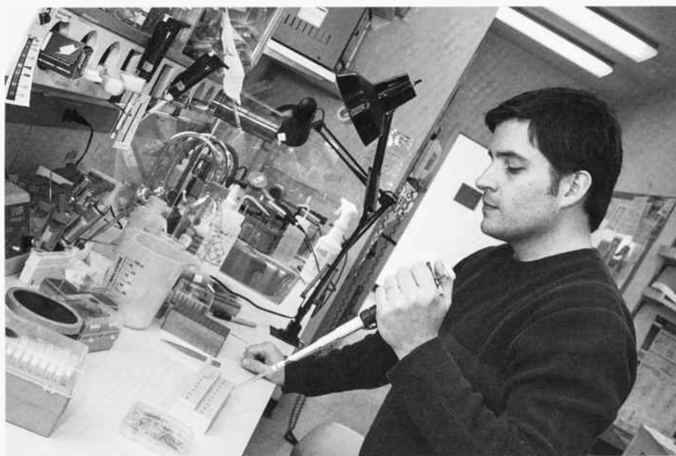

Fabio Nogueira, postdoc, Marja Timmermans' lab.

BIOINFORMATICS AND GENOMICS

Genome research has always involved the determination of the complete DNA sequences of organisms, the development of methods for assigning meaning to particular DNA, RNA, and protein sequences, and the exploration of how changes in gene expression govern normal biology and contribute to a variety of pathological states including cancer. As the fields of bioinformatics and genome research expand and mature, scientists are perfecting their ability to detect and decipher the fundamental patterns and other properties of the nucleic acids and proteins that ultimately shape all of life. This work involves the use of mathematics, statistics, and computer science to answer a wide variety of biological questions.

W. Richard McCombie's group, in collaboration with the Zhang, Martienssen, and Hannon labs, has developed a method to identify and verify the positions of genes within both plant and animal genomes. Using this method, the scientists discovered significant errors in what were considered "well-annotated" mouse genes. Similar work with rice showed that many of the rice genes that were considered full-length gene sequences in the rice genome database may instead represent only a fraction of the true number of rice genes.

Andrew Neuwald's group has developed software that detects subtle similarities between proteins of unknown function and proteins that have well-defined functions. Neuwald's group used the software to compare three distantly related protein families and to identify important structural similarities among the families that had previously been overlooked.

One of Lincoln Stein's interests includes creating software that compares DNA sequences and reveals common patterns that diverse organisms use to carry out similar life processes. For example, Stein has created software that enables scientists to compare the genomes of the major cereal crops (rice, wheat, corn, and oats) and thereby reveal beneficial genetic variants in one of these crops that might be exploited in the others. The results of these comparisons help plant breeders to develop new varieties with improved abilities to withstand disease, changes in climate (e.g., drought), and a variety of other adverse conditions. In other work that explored the genomic basis of "speciation" (i.e., the formation of new species), Stein took advantage of the newly completed genome sequence of the roundworm *C. briggsae*. (With some 500,000 species [after insects], roundworms comprise the second largest group of animals on Earth.) Stein's group compared the *C. briggsae* genome with that of the more commonly studied roundworm, *C. elegans*, both of which diverged from a common ancestor approximately 100 million years ago. Through this work, Stein and his colleagues have provided a comprehensive view of how genome evolution leads to the formation of new species.

Michael Zhang's group has developed a set of software tools for locating transcription-factor-binding site (TFBS) motifs—DNA sequences where gene regulators attach themselves and activate the series of events that lead to protein production. Obtaining a complete view of the types and locations of such binding sites, as Zhang's work enables, is crucial for understanding how and when genes are turned on and off in response to various biological and pathological cues.

GENOME SEQUENCE ANALYSIS

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

SEQUENCING OF THE RICE GENOME

This work was done in collaboration with Rod Wing (University of Arizona, Tuscon).

We have been collaborating with the Arizona Genome Institute (AGI) as part of a larger collaboration to sequence the genome of *Oryza sativa*, more commonly known as rice. The larger collaboration, the International Rice Genome Research Program (IRGSP), has been focused on completing a finished-quality sequence of the *Nipponbare* strain of rice. This is a strain of rice that is grown commonly in temperate regions such as Japan, as opposed to the more tropical strains of rice, such as *Indica*, which are more commonly grown in Southeast Asia. Several other large-scale sequencing projects have been under way by other groups on both *Nipponbare* and *Indica*. These groups, as well as ours, have produced and released draft-quality sequences of both the *Nipponbare* strain and an *Indica* strain. None of these sequences are of finished quality.

We and the other U.S. group, The Institute for Genomic Research, were funded to sequence chromosomes 3 and 10. We completed the sequence of chromosome 10 previously and were on schedule to complete chromosome 3 in 2004. It became clear, however, that several other groups around the world, whose completion schedule was moved forward due to competition, would be unlikely to complete their assigned regions in 2004. Moreover, a number of very difficult regions of the rice genome (such as heterochromatic sequences derived from pericentric regions) were particularly vexing for some of the groups participating in the project due to their relative lack of experience in dealing with such regions. As a result, we received additional funding and were assigned to finish regions on chromosomes 5, 9, and 11. We were also assigned the task of more globally attacking the remaining regions of gaps in the sequence. Finally, we were to carry out a training exercise in Asia, where we would work to provide additional training, particularly in dealing with difficult regions, to other finishers in the IRGSP.

In 2004, we completed on schedule our region of chromosome 3 and began the analysis of the region in preparation for a publication describing the sequence of that chromosome. We also completed our assigned regions on chromosomes 5, 9, and 11. These regions are also being analyzed. We participated in the global analysis of the genome as well. These analysis efforts and the publications ensuing from them will continue into 2005.

As part of this effort, we finished 10,689,516 bases in 2004. This ranged widely from month to month, with the early part of the year having much higher monthly totals since we were pushing to complete the bulk of the sequencing by the deadline. Since this was finished, we focused primarily on difficult clones, thereby reducing the amount of sequencing that was completed each month. We are still working on a small number of such clones.

In addition to BAC (bacterial artificial chromosome) clones assigned to the minimal tiling path of the rice genome by our collaborators at AGI, there are a number of clones (~2200) that are not assigned to a position in the genome. This happens in any large genome project, and the clones comprise a range of factors from contaminating DNA to legitimate clones that are too repetitive to map uniquely within the genome. To begin resolving these unassigned clones, we made four subclone libraries, each containing 96 pooled BAC clones, and we randomly sequenced 5244 of the subclones from each library. Analysis of the resulting sequence indicated that a high percentage of the sequence reads represented unique DNA that was probably derived from rice. As a result, we have begun sequencing a number of these clones individually at low coverage and finishing those clones that appear to be actual rice clones which had not been sequenced. This approach has already filled several gaps in the genome, and we will be continuing it into 2005.

We also successfully carried out the finishing training workshop. This workshop was patterned after segments of the postgraduate course in DNA sequencing that we taught at CSHL for a number of years. The

course was conducted in conjunction with the Rice Genome Forum in Tsukuba, Japan. The Japanese Rice Genome Program and the Society for Techno-Innovation of Agriculture, Forestry, and Fisheries collaborated with us in setting up and running the course. Representatives from five different centers (four different countries) participated.

ANALYSIS OF TRANSCRIPTIONAL START SITES IN MOUSE GENES

This work was done in collaboration with Michael Zhang and Greg Hannon both here at CSHL. We have become very interested in the composition of the mammalian transcriptome. This is a fundamental question, and we are concerned that current work fails to recognize substantial numbers of genes as well as to accurately describe the transcriptional start site and alternatively spliced variants of many of these genes.

As a first step in addressing this problem, we sought to analyze the accuracy of a number of gene annotations in the mouse. These annotations ranged from those to which one would a priori assign a high level of confidence to gene predictions that are not supported by any experimental evidence and are not considered in the canonical gene set.

A total of 300 gene predictions or annotated genes were selected for analysis and grouped into five different categories on the basis of the quality and quantity of supporting evidence for the gene model. These gene categories included well-characterized genes in the Eukaryotic Promoter Database (EPD category), genes from the RefSeq gene set (RefSeq category), automated gene predictions which are linked to multiple expressed sequence tags (ESTs)/mRNAs (category B), as well as computational predictions with a single EST match (category C) or no extant empirical support (category D). The results of RACE-PCR (polymerase chain reaction) amplification of the 5′ ends of these genes in 15 pooled mouse tissues/embryonic stages are summarized in Table 1. Modifications were made to the 5′ RACE-PCR procedure, allowing pooling of RNA samples, resulting in a scaleable procedure.

The results illustrate potential errors or omissions in the current 5′-end annotations in 58% of the genes detected. In testing experimentally unsupported gene predictions (categories C and D), we were able to identify 58 that are not in the canonical gene set but produced spliced transcripts (~25% success rate). In addition, in many genes, we were able to detect novel exons not predicted by any gene prediction algorithms. In 19.8% of the genes detected in this study, multiple transcript species were observed. These data show an urgent need to provide direct experimental validation of gene annotations. Moreover, these results show that direct validation using RACE-PCR can be an important component of genome-wide validation. This approach can be a useful tool in the ongoing efforts to increase the quality of gene annotations, especially transcriptional start sites, in complex genomes.

TABLE 1 Description of gene categories and analysis of 5′ end annotations

Category	No. of Genes in category	No. of Genes successfully amplified by 5′ RACE (%)	No. of RACE sequences that differed from the 5′ gene annotation (%)
EPD	13	13 (100%)	4 (31%)
RefSeq	27	20 (74%)	8 (40%)
B	23	15 (65%)	7 (47%)
C	169	40 (24%)	30 (75%)
D	68	18 (26%)	12 (67%)
Totals	300	106 (35%)	61 (58%)

SEQUENCING OF shRNAi LIBRARIES

We have been collaborating with the Hannon lab here at CSHL to make large-scale libraries containing short hairpin RNAi clones targeted to the human and mouse genome. These will be described more completely in Greg Hannon's annual report, but we wanted to describe the sequencing of the libraries in our report.

The most cost-effective way to make the libraries is in a random fashion. This greatly reduces the cost of oligonucleotides but also requires sequencing large numbers of random clones and selecting those with the correct sequence. A side benefit of this approach is that all of the clones in the library are sequence-verified.

Sequencing of these libraries required us to modify our protocols since we wanted short read lengths (hence, more runs per day per machine), and the hairpin structure of the clones was problematic. We made these modifications and developed a high-throughput pipeline for sequencing these clones. In 2004, we sequenced 652,609 hairpins for this project.

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Fred Katzenberger, lab tech, Dick McCombie's lab.

OBTAINING MECHANISTIC CLUES FROM THE EVOLUTIONARY CONSTRAINTS IMPOSED ON PROTEIN SEQUENCES

A.F. Neuwald N. Kannan

The selective constraints acting upon proteins during evolution are revealed in multiple sequence and structural alignments as coconserved residues and atomic interactions. These patterns of conservation contain implicit information about underlying protein mechanisms, just as crystalline X-ray diffraction patterns contain implicit information about a protein's structure. Our previous research led to the development of an approach called contrast hierarchical alignment and interaction network (CHAIN) analysis, which measures and characterizes selective constraints based on coconserved patterns and then fits these constraints, in light of other biological information, to possible mechanistic models for protein function. Just as the quantity and quality of X-ray diffraction patterns determines how well one can fit an atomic model to the inferred electron density, the quantity and quality of coconserved patterns determine how well one can fit possible mechanisms to inferred selective constraints. In principle, given sufficient data, biological mechanisms may be identified with fairly high certainty. Nevertheless, even at "low resolution," these inferred constraints can prune the possibilities down to a manageable number. Moreover, because natural selection imposes constraints on whole organisms within their native environments, these analyses lack the artifactual biases sometimes associated with in vitro experimental systems or with in vivo cell cultures. Furthermore, they can reveal functionally critical features that biologists have entirely overlooked due to inherent limitations of current experimental methods. In this way, our research taps into nonhypothesis-driven (sequence, structural, and taxonomic) experimental data to generate working hypotheses that can fuel further experimentation.

This year, we have continued to develop new methods and to apply them mainly to classes of proteins for which vast amounts of sequence, structural, and other experimental data are available, in which case, powerful statistical procedures can be used to detect subtle yet significant characteristics. We continue to focus on biomedically important proteins, such as protein kinases, which are key targets for cancer therapies.

Multiple Alignment through Monte Carlo Optimization of a Hidden Markov Model

A. Neuwald [in collaboration with J. Liu, Department of Statistics, Harvard University]

Our ability to quantify selective constraints strongly depends on the quality of our multiple alignments, which thus motivated development of theoretical concepts and strategies to improve alignment of conserved regions within large sets of distantly related sequences. This work, which was published this year, describes a hidden Markov model (HMM), an algebraic system, and Markov chain Monte Carlo (MCMC) sampling strategies for alignment of multiple sequence motifs. The MCMC sampling strategies are useful both for alignment optimization and for adjusting our measures of selective pressure for alignment uncertainties. These also use powerful Bayesian statistical procedures that provide an objective measure of alignment quality as well as automatic gap penalty optimization and that thus can distinguish very subtle, yet significant sequence conservation from random "noise." In contrast, essentially all currently widely used multiple alignment programs, such as ClustalW and T-coffee, and newer "state-of-the-art" programs, such as MUSCLE and MAFFT, are unsuitable for statistical analysis because these align randomly generated sequences (implying that they easily confuse noise with biologically valid conservation). Thus, our new methods often more accurately align very distantly related sequences and thereby provide a better measure of selective constraints. Programs implementing these methods have been made available, including GISMO (Gibbs sampling with multiple operations) and GARMA (genetic algorithm for recombinant multiple alignment).

Analysis of Eukaryotic Protein Kinases

N. Kannan, A. Neuwald

An analysis of coconserved residues associated with functional specificity of cyclin-dependent kinases

(CDKs), mitogen-activated protein kinases (MAPKs), glycogen synthase kinases (GSKs), and CDK-like kinases (CLKs), which are collectively termed the CMGC group, was published this year. Many of the inferred functional constraints shared by and specific to CMGC kinases correspond to a region between the start of an activation loop, which undergoes a conformational change upon phosphorylation to turn on these kinases, and a CMGC-conserved insert segment associated with coprotein binding. The strongest such constraint is imposed on a "CMGC-arginine," the side chain of which has a role both in substrate recognition and kinase activation. These and other CMGC features suggest a functional association between coprotein binding, substrate recognition, and kinase activation. Constraints shared by and specific to individual subfamilies point to mechanisms for kinase specialization and provide direction for design of drugs with high specificity. We also have extended our analysis of kinases further by using the GISMO and GARMA programs to align, statistically analyze, and compare eukaryotic protein kinases (EPKs) with distantly related EPK-like kinases and with very distantly related atypical protein kinases. This has revealed an ancient conserved component that appears to couple substrate phosphorylation to nucleotide exchange—an inherent mechanism that seems to have been recruited by EPK regulatory components during evolution. EPKs phosphorylate constituents of various cellular signaling pathways and thus must be tightly regulated to avoid physiological catastrophes.

Analysis of AAA+ ATPases

A. Neuwald

We continue to study aspects of AAA+ ATPase mechanisms. These proteins are chaperones involved in the assembly, operation, and disassembly of multiprotein complexes. Our current emphasis is on DNA polymerase clamp loader complexes, which load onto DNA and unload a clamp that links the replication complex to DNA during polynucleotide synthesis. Last year, we initiated an analysis of prokaryotic DNA polymerase III γ and δ' clamp loader subunits and published an analysis of corresponding β-clamps. This year we have extending our analysis of clamp loaders to eukaryotic replication factor C (RFC) subunits. These studies have required further refinement of various CHAIN analysis procedures, including the development of a multiple alignment program that utilizes curated alignments—one for each AAA+ family and based on detailed motif analyses and structural comparisons. This program allows us to obtain very accurate alignments of proteins sharing only weakly conserved motifs separated by highly variable inserts (as for the AAA+ class). Likewise, we have developed methods for measuring precise changes in selective constraints between closely related protein families whose functions differ only slightly. These measures are particularly relevant to analysis of certain eukaryotic RFC clamp loader ATPases, because, unlike their bacterial counterpart (the γ subunit), these have diversified into three distinct families of ATPases having subtle functional distinctions. By quantifying the differences in the selective constraints imposed on these proteins, we thus were better able to decipher specific aspects of their underlying mechanisms.

Expanding into Other Protein Classes

A. Neuwald

We also have initiated CHAIN analyses of additional key protein constituents of the cellular machinery. This was reported as preliminary studies for a recently submitted National Institutes of Health grant proposal to expand our analyses to many other protein classes and to create a database of the coconserved structural components thus identified. These studies involved about a dozen domain classes including superfamily 1 and 2 helicases, ATP-binding cassette (ABC) ATPases, RNA recognition motifs (RRMs), the actin/HSP70/sugar kinase superfamily, amino acid tRNA synthetases, α,β-hydrolase fold enzymes, pyridoxal phosphate-binding proteins, dehalogenases, FAD/NAD-binding proteins, N-acetyl transferases, methyltransferases, histone fold proteins, histidine kinases, and helix-turn-helix-binding domains. Some of these classes are vast and well characterized and thus are highly amenable to analysis. Several of these proteins are of interest to other CSHL research groups, who plan to follow up experimentally on hypotheses generated by this project. Drs. Tonks and Muthuswamy here at CSHL are currently following up on certain preliminary analyses of protein phosphatases and kinases, respectively.

A CHAIN Analysis Database

A. Neuwald, N. Kannan [in collaboration with L. Stein, Cold Spring Harbor Laboratory]

Work on our proposed CHAIN analysis database thus far has involved setting up "contrast heirarchical alignment" files and files for rendering three-dimensional structural views of specific proteins. These files will be created for each published analysis. Each alignment file represents a particular category of selective constraints imposed on a query sequence, which corresponds to the protein whose structure is shown in the corresponding three-dimensional rendering file. The three-dimensional rendering file uses a color scheme to represent the various categories of selective constraints. These and other files will be made available over the World Wide Web to allow researchers to visualize the coconserved structural components, inferred selective constraints, and proposed molecular mechanisms coming out of our analyses.

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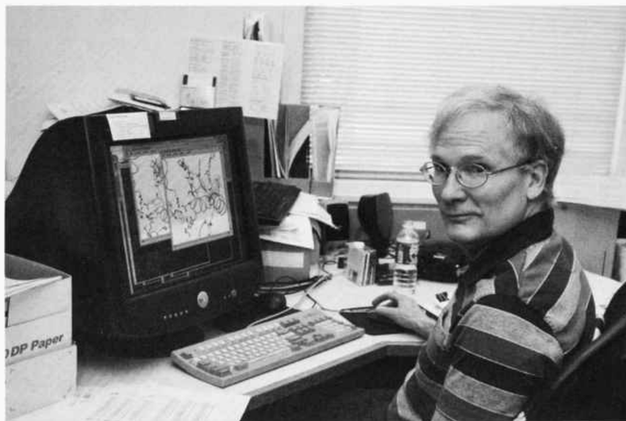

Andy Neuwald

COMMUNITY ACCESS TO GENOME RESOURCES

L. Stein S. Avraham S. Cain P. Canaran N. Chen B. Faga T. Fiedler M. Gillespie G. Gopinathrao T. Harris B. Hurwitz G. Joshi-Tope L. Krishnan K. Kumar C. Liang C. Maher L. Matthews S. McKay L. Ren S. Schmidt A.V. Smith W. Spooner G. Thorisson P. van Buren G. Wu C. Youens-Clark W. Zhang

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. During this year, we developed a new integrated map of the rice and maize genomes that allows researchers to move back and forth between various genetic and physical maps of these species in order to apply the knowledge developed in one organism to finding functionally significant genes in the other.

Curation is an important component of Gramene. In collaboration with Susan McCouch's laboratory at Cornell, we have curated more than 28,000 rice proteins, assigning them classifications in the Gene Ontology (www.geneontology.org). In addition, we have classified nearly 1000 rice mutants using a trait ontology that we have developed. We are currently curating the rice biological literature, much of which is in non-English languages, and making this information available via Gramene as well.

During the past year, Gramene increased its holding substantially by adding a variety of important comparative mapping resources, including high-throughput gene-rich sequences from maize, sorghum, rye grass, and a series of mapped transposon insertions useful for functional genomics. We have also begun a project to reconstruct the genomes of 11 strains of wild rice that include multiple independent polyploidization events. This will be a unique opportunity to understand the biological basis of this agriculturally important phenomenon.

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

During 2004, we have enhanced WormBase by adding mapping and sequencing information from the genome of *C. briggsae*, a sister species of *C. elegans* that diverged approximately 100 million years ago. This information allowed us to discover and confirm a new family of chemosensory (olfactory) receptor genes, which culminated in a publication in the

Proceedings of the National Academy of Sciences. We have also added considerable new functional information to WormBase, including a large protein-protein interaction data set and SAGE (serial analysis of gene expression) data. These data set the stage for a systems biology approach to *C. elegans*, in which the community reconstructs the control circuits of the nematode's genetic regulatory pathways. Another WormBase-related project that is under way is the sequencing of four additional *Caenorhabditis* nematodes. These genomes will be available from WormBase beginning in the spring of 2005.

REACTOME

Reactome (www.reactome.org; formerly "Genome Knowledgebase") 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 approximately 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 is 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 2004, we completely revamped the Web site to create an intuitive "birds eye view" of human metabolism (see Fig. 1). Reactome received favorable

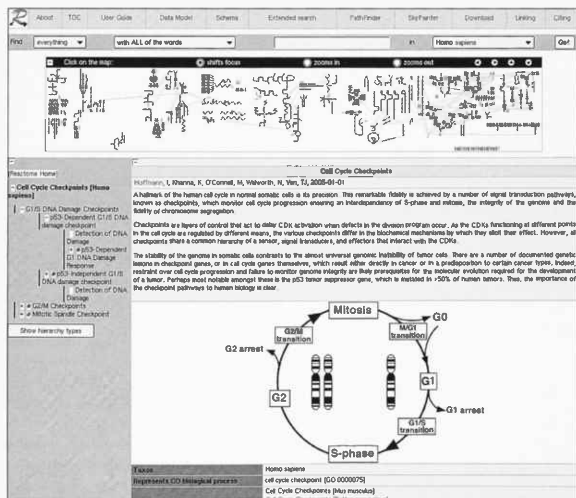

FIGURE 1 Visualizing pathways with Reactome. This shows a page from the Cell Cycle Checkpoints module. At the top is a graphical view of all pathways in Reactome. Those pathways that are involved in cell cycle checkpoints are highlighted in dark blue in the color Web page (www/Reactome.org). In the middle of the page is a human-readable summary of the process and a diagram. At the bottom are fields from the database that list all the proteins and complexes known to be involved in the process and provide access to orthologous events in other vertebrates. By clicking on the expandable outline on the left, the user can drill down into increasingly detailed pages.

reviews in *Science*, *Nature*, and other high-profile journals, and an article about the resource will appear in the January 2005 edition of *Nucleic Acids Research*.

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.

THE HUMAN HAPLOTYPE MAP

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

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 2004, we greatly enhanced the Web site by adding data visualization and analysis tools based on the GMOD infrastructure. In particular, we added a mechanism for viewing patterns of linkage disequilibrium within a population and for examining the differences among populations. We also provided a mechanism for downloading selected sets of genotypes in a format that can be used for haplotype block analysis.

As of late 2004, the project had nearly reached its target density of 1 genotyped SNP every 5 kb, and a publication describing patterns of linkage disequilibrium is in preparation. However, in light of the potential utility that it has already shown, the project has recently been extended to genotype an additional 2.5 million SNPs and the project is slated to continue through 2005.

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COMPUTATIONAL GENOMICS

M.Q. Zhang N. Banerjee N. Dimitrova L. Liu P. Sumazin
G.X. Chen N. Hata D. Schones J.H. Wang
D. Das C. Jiang A.D. Smith Z.Y. Xuan
R. Das M. Kato S. Srivastava F. Zhao

Our main achievement last year was to continue building our own large-scale genome analysis infrastructure. We further developed our promoter annotation pipeline to include manually curated information on cancer-related transcription factors, their binding sites, and target gene regulation. Most significantly, we developed a whole suite of novel computational algorithms/tools for identification of transcription-factor-binding site (TFBS) motifs by taking all three large-scale genomics resources: genomic DNA sequences, ChIP-chip, and expression microarray data. First of all, we clustered the existing known TFBSs in *TRANSFAC* and *JASPER* and developed a rigorous distance measure for measuring similarity between any given pair of motifs using *Matcompare*. Second, we developed a pair of novel discriminant motif finders, called *DWE* (discriminant word enumerator) and *DME* (discriminant matrix enumerator), which allows users to identify differentially "expressed" motifs between two arbitrary (target and control) sequence sets. Third, we developed a *MARS* (multivariate adaptive regression splines)-based motif finder *MARSMotif*, which allows users to automatically select functionally significant motifs and motif combinations by correlating this sequence information with microarray data. These tools have been successfully tested to find tissue-specific motifs in liver and in muscle when combining sequence information with microarray data. We are in the process of integrating these resources to systematically and automatically identify a more comprehensive list of tissue-specific TFBS motifs and motif combinations (modules) in the human genome.

TRED: A Transcriptional Regulatory Element Database and a Platform for In Silico Gene Regulation Studies

F. Zhao, Z.Y. Xuan, L. Liu, M.Q. Zhang

To understand gene regulation, accurate and comprehensive knowledge of transcriptional regulatory elements is essential. Here, we report our efforts in building a mammalian transcriptional regulatory element database (TRED) with associated data analysis functions. It collects *cis*- and *trans*-regulatory elements and is dedicated to easy data access and analysis for both single-gene-based and genome-scale studies. Distinguishing features of TRED include (1) relatively complete genome-wide promoter annotation for human, mouse, and rat; (2) availability of gene transcriptional regulation information including TFBS and experimental evidence; (3) data accuracy ensured by hand curation; (4) efficient user interface for easy and flexible data retrieval; and (5) implementation of on-the-fly sequence analysis tools. TRED can provide good training data sets for further genomewide *cis*-regulatory element prediction and annotation, assist detailed functional studies, and facilitate the decipher of gene regulatory networks (http://rulai.cshl.edu/TRED).

Similarity of Position Frequency Matrices for TFBSs

D. Schones, P. Sumazin, M.Q. Zhang

TFBSs in promoter sequences of higher eukaryotes are commonly modeled using position frequency matrices (PFMs). The ability to compare PFMs representing binding sites is especially important for de novo sequence motif discovery, where it is desirable to compare putative matrices to one another and to known matrices. We have described a PFM similarity quantification method based on product multinomial distributions, demonstrated its ability to identify PFM similarity, and shown that it has a better false-positive to false-negative ratio compared to existing methods. We grouped TFBS frequency matrices from two libraries into matrix families and identified the matrices that are common and unique to these libraries. We identified similarities and differences between the skeletal-muscle-specific and non-muscle-specific frequency matrices for the binding sites of Mef-2, Myf,

Sp-1, SRF, and TEF of Wasserman and Fickett (*J. Mol. Biol. 278:* 167–181 [1998]). We further identified known frequency matrices and matrix families that were strongly similar to the matrices given by Wasserman and Fickett. We provided methodology and tools to compare and query libraries of frequency matrices for TFBSs.

DWE: Discriminating Word Enumerator

P. Sumazin, G.X. Chen, N. Hata, A.D. Smith, M.Q. Zhang
[in collaboration with T. Zhang, Merck Research Laboratories, Boston]

Tissue-specific TFBSs provide insight into tissue-specific transcription regulation. We have described a word-counting-based tool for de novo tissue-specific TFBS discovery using expression information in addition to sequence information. We incorporated tissue-specific gene expression through gene classification to positive expression and repressed expression. A direct statistical approach was used to find overrepresented TFBSs in a foreground promoter sequence set against a background promoter sequence set. Our approach naturally extends to a synergistic TFBS search. Putative TFBSs were found overrepresented in the proximal promoters of liver-specific genes relative to proximal promoters of liver-independent genes. Our results indicate that binding sites for hepatocyte nuclear factors (especially HNF-1 and HNF-4) and CCAAT/enhancer-binding protein (C/EBPbeta) are the most overrepresented in proximal promoters of liver-specific genes. The results also suggest that HNF-4 has strong synergistic relationships with HNF-1, HNF-4, and HNF-3beta and with C/EBPbeta.

Identifying Tissue-selective TFBSs in Vertebrate Promoters with *DME*

A.D. Smith, P. Sumazin, M.Q. Zhang

We demonstrated a computational method, *DME* (discriminant matrix enumerator), aimed at systematically identifying tissue-selective TFBSs. Our method focuses on the differences between sets of promoters that are associated with differentially expressed genes, and it is effective at identifying the highly degenerate motifs that characterize vertebrate TFBSs. Results on simulated data indicate that our method detects motifs with greater accuracy than the leading methods, and its detection of strongly overrepresented motifs is nearly perfect. We demonstrated motifs identified by our method as the most overrepresented in promoters of liver- and muscle-selective genes, showing that our method accurately identifies known TFBSs and previously uncharacterized motifs.

Using *MARS* to Study Interacting Models of Cooperative Gene Regulation

D. Das, N. Banerjee, M.Q. Zhang

Cooperation between transcription factors is critical to gene regulation. Current computational methods do not take adequate account of this salient aspect. To address this issue, we used a computational method based on multivariate adaptive regression splines (MARS) to correlate the occurrences of transcription-factor-binding motifs in the promoter DNA and their interactions with the logarithm of the ratio of gene expression levels. This allows us to discover both the individual motifs and synergistic pairs of motifs that are most likely to be functional and to enumerate their relative contributions at any arbitrary timepoint for which mRNA expression data are available. We present results of simulations and focus specifically on the yeast cell cycle data. Inclusion of synergistic interactions can increase the prediction accuracy over linear regression to as much as 1.5- to 3.5-fold. Significant motifs and combinations of motifs are appropriately predicted at each stage of the cell cycle. We believe our multivariate adaptive regression splines-based approach will become more significant when applied to higher eukaryotes, especially mammals, where cooperative control of gene regulation is absolutely essential. Our integrated software tool is called *MARSMotif.*

Computational Dissecting Transcriptional Regulation Network in Yeast Cell Cycle

M. Kato, N. Hata, N. Banerjee, M.Q. Zhang

Combinatorial interaction of transcription factors (TFs) is important for gene regulation. Although vari-

ous genomic data sets are relevant to this issue, each data set provides relatively weak evidence on its own. Developing methods that can integrate different sequence, expression, and localization data have become important. We use a novel method that integrates chromatin immunoprecipitation (ChIP) data with microarray expression data and with combinatorial TF-motif analysis. We systematically identify combinations of transcription factors and motifs. The various combinations of TFs involved multiple binding mechanisms. We reconstruct a new combinatorial regulatory map of the yeast cell cycle in which cell cycle regulation can be drawn as a chain of extended TF modules. We find that the pairwise combination of a TF for an early cell cycle phase and a TF for a later phase is often used to control gene expression at intermediate times. Thus, the number of distinct times of gene expression is greater than the number of transcription factors. We also see that some TF modules control branch points (cell cycle entry and exit), and in the presence of appropriate signals, they can allow progress along alternative pathways. Combining different data sources can increase statistical power as demonstrated by detecting TF interactions and composite TF-binding motifs. The original picture of a chain of simple cell cycle regulators can be extended to a chain of composite regulatory modules: Different modules may share a common TF component in the same pathway or a TF component cross-talking to other pathways.

From Worm to Human: Bioinformatics Approaches to Identify FOXO Target Genes

Z.Y. Xuan, M.Q. Zhang

Longevity regulatory genes include the Forkhead transcription factor FOXO, in addition to the NAD-dependent histone deacetylase silent information regulator 2 (Sir2). The FOXO/DAF-16 family of transcription factors constitutes an evolutionarily conserved subgroup within a larger family known as winged helix or Forkhead transcriptional regulators. In a study of aging, we have demonstrated how to identify FOXO target genes and their potential *cis*-regulatory binding sites in the promoters via bioinformatics approaches. These results provide new testable hypotheses for further experimental verifications.

Evidence and Characteristics of Putative Human Alpha Recombination Hot Spots

M.Q. Zhang [in collaboration with X.G. Zhang, Tsinghua University, China]

Understanding recombination rate variation is very important for studying genome diversity and evolution, and for investigation of phenotypic association and genetic diseases. Recombination hot spots have been observed in many species and are well studied in yeast. Recent study has demonstrated that recombination hot spots are also a ubiquitous feature of the human genome. But the nature of human hot spots remains largely unknown. We have developed and validated a novel computational method for testing the existence of hot spots as well as for localizing them with either unphased or phased genotyping data. To study the characteristics of hot spots within or close to genes, we scanned for unusually high levels of recombination, using the European population samples in the *SeattleSNPs* database, and found evidence for the existence of human alpha hot spots similar to those of yeast. This type of hot spot, found at promoter regions, accounts for about half of the total detected and appears to depend on some specific transcription-factor-binding sites (such as CGCCCCCGC). These characteristics can explain the observed weak correlation between hot spots and GC content, and their variation may contribute to the diversity of hot spot distribution among different individuals and species. These long-sought putative human alpha recombination hot spots deserve further experimental investigations.

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Members of Michael Zhang's lab.

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 Grossniklaus (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 Bensimon and Vincent Croquette. Terence is using single-molecular biophysics to study the mechanical response of DNA to stretching and twisting by enzymes that alter DNA topology, thus elucidating the properties of those enzymes. Terrence left last year 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 this year. Their reports are listed below. Lee joined us from Doug Melton's laboratory at Harvard University where he earned his Ph.D. for studies on *Xenopus* development. Lee is studying taste bud development and the regulation of taste-bud-specific genes. 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 plans to use this library to determine the genes that are required for specification of embryonic stem cell fates during differentiation. 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 examining the potential role of copy-number polymorphisms in controlling genetic variation.

Investigation of DNA Copy-number Fluctuation using Genomic Microarrays

I. Hall

We are interested in pursuing a genome-wide examination of genetic instability and plasticity and in characterizing the contribution of DNA copy-number polymorphisms 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. During the next few years, we plan to use the laboratory mouse as a model system to investigate the frequency and nature 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 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 on 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 DNA copy-number polymorphisms (CNP) and restriction-fragment-length polymorphisms (RFLP) 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 requires only that a complete genome sequence exists for the organism in question. Using ROMA, the Wigler group here at CSHL has recently discovered extensive copy-number polymorphism between individual humans (Sebat et al., *Science 305:* 525 [2004]), indicating that CNPs may account for a substantial fraction of human genetic variation.

The apparent evolutionary importance and contemporary prevalence of segmental copy-number variation raises fundamental questions: How often do new CNPs arise? Do different tissues or cellular lineages differ in their susceptibility to genetic alteration? Are different chromosomal regions, classes of DNA sequence, or gene families more variable than others? Are there genetic pathways or environmental conditions that compromise genome stability? Do different cells or cell types of an organism always have the same DNA content?

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 obvious experimental advantages of controlled crosses, genomic manipulation, and mutant analysis. Furthermore, 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.

The work outlined below relies heavily upon technology, infrastructure, and expertise developed in the Wigler and Lucito laboratories here at CSHL during the past few years.

HOW GENETICALLY DIVERSE ARE LABORATORY MICE?

To get a broad picture of the current genetic composition of the laboratory mouse and the utility of ROMA as a tool for mouse genetics, we have compared a handful of different commonly used strains to a reference. These preliminary experiments show that a large number of ROMA polymorphisms exist among different inbred strains (3–5% of probes) and that these polymorphisms are distributed throughout the genome in a punctate, nonrandom fashion that presumably reflects the ancestral origin of chromosome segments. The majority of these polymorphisms appear to represent variation existing before the separation of inbred lines, and rough estimates suggest that a significant portion of these are CNPs (~10–15%). In general, these polymorphic markers have only two variants, indicating that the original genetic pool giving rise to modern strains was very limited. Statistical interpretation of these patterns requires new algorithms, and we plan to develop these in collaboration with M. Wigler. These tools, and a knowledge of the differences in haplotype distribution and gene copy number between strains, will represent a major experimental resource for future studies.

The oldest inbred lines have been propagated by brother-sister mating for more than 200 generations and are thought to be genetically uniform. Our pilot experiments comparing separate individuals from various strains indicate that although mice within a given strain are highly inbred, they are not identical. They do not retain ancestral polymorphisms (those that distinguish strains) but do exhibit an unexpected number of unique CNPs that presumably have arisen recently and have not yet been fixed within, or lost from, the inbreeding lineage. Some of these CNPs contain well-known genes. Our experiments thus far are not sufficient in scale to gauge the extent to which unique CNPs are segregating within otherwise inbred lines, but they do suggest a relatively high rate of de novo formation.

HOW COMMON ARE GENOME REARRANGEMENTS?

To assess the importance of segmental duplication and deletion in generating de novo genetic variation, we plan to examine 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. These studies will also provide the necessary context for future work characterizing molecular pathways and environmental stimuli that promote, or inhibit, genome stability through a direct effect on chromosome behavior.

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. We have initiated studies to estimate these parameters by analyzing the frequency at which new CNPs arise in families of mice.

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, P. O'Brien

The goal of our research is to understand the molecular basis of vertebrate taste receptor cell genesis. These specialized sensory cells are housed within ovoid structures called taste buds, which are embedded in the epithelium of the tongue, and to a lesser extent the epithelium of the palate and upper pharynx. Consisting of 70–100 cells, the vertebrate taste bud is a highly dynamic structure that possesses both epithelial and neuronal qualities. Like all other sensory cell types, the taste receptor cell membrane depolarizes in the presence of a suitable stimulus. Similar to the epithelial cells that line the intestine and other areas of the gut, taste receptor cells turn over at a rapid rate (~8–10 days in rodents). Thus, 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. Unlike the olfactory system, where the axons of newly formed receptor cells project for some distance back toward and synapse on to neurons in the glomeruli of the olfactory bulb, newly formed taste receptor cells lack classical projections and synapse on to sensory afferents that are associated with the taste bud.

The basic goal of our research is to understand how the daughters of the progenitors select a particular fate. Because different receptor cells are known to respond to different classes of tastants, we hope that understanding this differentiation process will help to illuminate details of both taste bud structure and function. Toward this end, we have undertaken an exhaustive effort to define at the level of transcription the cellular diversity of the murine taste bud. To do this, single-cell profiling is being used to analyze the transcriptomes of taste receptor and progenitor cells.

A prerequisite for much of our work is the development of a quantitative method for the analysis of mRNA expression in single or small numbers of cells. A typical eukaryotic cell contains picogram amounts of mRNA, whereas the vast majority of the analytical procedures that we use to analyze gene expression require microgram amounts of material. Thus, the mRNA expressed in a single cell must be amplified before it can be studied. We have developed a novel scheme for performing such amplifications. In this technique, mRNA is bound to a magnetic bead, covalently coupled cDNA is produced, and after a series of molecular manipulations, the cDNA is released and amplified by either PCR (polymerase chain reaction) or in vitro transcription.

We initially struggled with severe signal-to-noise problems using this system, which was expected because similar but solution-based assays have similar issues. This problem has been largely eliminated through the use of a novel oligonucleotide design, and during the previous year, we have concentrated on the chemistry involved in linking the capture/probe

oligonucleotide to the magnetic bead. The linker must have three properties: covalent attachment to the bead surface, the ability to cleave oligomer/CNDA free from the bead in a specific manner, and sufficient length to position the oligomer far from the bead. All of these requirements have been met through the synthesis of a 12–24-unit polyethylene glycol (PEG)-containing linker that possesses a free amine for attachment to carboxyl functionalized magnetic beads on one end and a *t*-butyl carbamate (BOC)-protected amine on the other. Proximal to the BOC-protected amine is a *cis* diol that can be specifically cleaved by periodate oxidation in aqueous conditions. Once attached to the bead, the BOC-protecting group is removed and the free amino acylated with a commercially available adapter that allows the linker to form a stable thioether bond with 5′ thiol-modified oligonucleotides. We are currently testing the effectiveness of our background reduction method utilizing beads covalently attached to oligonucleotides through the aforementioned linker. We will also access the degree to which the linker influences the kinetics of both mRNA hybridization and the various enzymatic manipulations that are needed to convert the mRNA into cDNA.

Protein–DNA Interactions

T.R. Strick

Because my laboratory has just moved to Paris, France, this is my last Annual Report as a Cold Spring Harbor Laboratory Fellow. I would like to thank the Laboratory for the excellent working conditions, exciting atmosphere, and generous suppport it has provided me during the last four years. It has been a great privilege to spend time as a part of the Cold Spring Harbor Laboratory community.

This past year, we have made exciting progress in our efforts to detect protein–DNA interactions in real time. In particular, we have been using single-molecule nanomanipulation techniques to modify and study the structure and topology of DNA in processes ranging from gene transcription to the packaging of DNA into mitotic chromosomes. These methods yield important new mechanistic insights into these molecular motors and complex systems that transcribe the genome or maintain its integrity. At the same time, they also shed light on the nature of DNA structural transitions and their influence on gene expression.

DNA nanomanipulation allows us to observe and control protein–DNA interactions at the resolution of individual molecules. Continuous monitoring of a single pair of interacting molecules (for instance, an RNA polymerase molecule and a DNA molecule) gives us access to the full time course of the interaction, thereby allowing us to directly observe the interaction in real time—something that is simply not possible using traditional biochemistry and molecular biology. From this, one can measure with unprecedented accuracy and precision the time scales of interactions between the protein and its DNA substrate and perform quantitative analysis of, for instance, association and dissociation rates, and their dependence on activators (such as CAP protein) or inhibitors (such as antibiotics).

In addition, nanomanipulation of the DNA allows us to control the level of mechanical strain (i.e., supercoiling) applied to it. In this way, we can dynamically and quantitatively reproduce in vitro the mechanical constraints to which DNA is thought to be subjected to in vivo. This allows us to elucidate mechanical features of the interaction, such as how protein binding induces twisting, bending, or looping of the DNA. It also allows us to determine how the protein–DNA interaction responds to changes in DNA topology and strain. Such experiments help us to better understand how many proteins function—by changing their and their substrates' conformations.

The single-molecule experiment we have implemented is depicted in Figure 1. A 2-kb linear DNA molecule (~0.6 μm long) is shown anchored at one

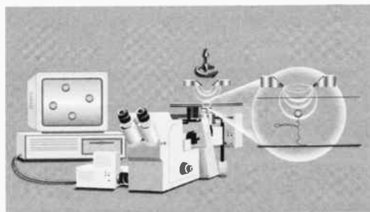

FIGURE 1 Depiction of the experimental setup. (*Inset*) DNA is tethered at one end to the wall of a glass capillary tube and at the other to a magnetic bead. (*Overview*) The capillary tube is mounted on an inverted microscope whose focus is controlled by a computer. The computer also controls the displacements (translation and rotation) of the magnets used to manipulate the bead. A CCD camera connected to the microscope relays video images of the magnetic bead to the computer. The computer extracts from these images the mean position and the Brownian fluctuations of the bead, which can be used to determine the DNA's end-to-end extension l, which results from an applied stretching force F and a supercoiling of n turns.

end to a glass surface and at the other end to a 1-μm-diameter magnetic bead. The field generated by magnets located above the sample is used to pull on and rotate the magnetic bead, thus stretching and twisting the tethered DNA. The stretching force applied to the DNA via the bead is calibrated as a function of magnet position (the closer the magnets, the higher the force). The torsion imparted to the DNA is exactly equal to the number of clockwise or counterclockwise rotations performed by the magnets and imposed on the bead. The double helix is thus quantitatively and reversibly supercoiled while held under tension. By determining the position of the magnetic bead, one measures and calibrates the end-to-end extension of the DNA molecule, and thus its mechanical response to stretching and twisting.

Single-molecule Studies of Transcription Initiation

A. Revyakin, R.H. Ebright, T.R. Strick

The transcription of DNA (gene) into RNA (message) is the first step in the regulation of gene expression. Transcription begins when RNA polymerase binds to the promoter sequence, which heralds the beginning of a gene. The enzyme unwinds the DNA at the promoter site (forming a "transcription bubble") to better read the sequence of base pairs encoding the gene. It then begins to synthesize short RNA fragments as it attempts to break free from the promoter site, in an apparently futile process known as "abortive initiation." Only after several rounds of abortive initiation will the enzyme be able to break free from the promoter site and begin productive elongation of the RNA transcript. After the end of the gene and upon reaching the "termination sequence," the RNA polymerase releases the RNA transcript and itself dissociates from the DNA.

We have recently made significant progress in using single-DNA nanomanipulation to perform real-time studies of the interactions between RNA polymerase and DNA. These experiments allow us to observe a single RNA polymerase molecule as it works to transcribe a single DNA molecule. In the past, our research had focused on the first few steps of transcription, in particular the mechanical unwinding of the promoter site by RNA polymerase (Fig. 2A).

In the last year, technical improvements to our nanomanipulation system have allowed us to increase our temporal resolution by at least a factor of 2, thereby making it possible to detect RNA polymerase/DNA interactions on a time scale of a few hundred milliseconds. We have exploited this improvement to extend our measurements of RNA polymerase/DNA interactions to all of the events that follow transcription initiation, i.e., abortive initiation, promoter escape, productive elongation, and the termination of transcription. We are thus able to observe in real time the entire transcription cycle, detecting one RNA polymerase after another as it completes its transcription "burst" on the DNA molecule that we control by nanomanipulation.

In Figure 2B, we present data showing transcription of the nanomanipulated DNA by four successive RNA polymerase molecules. Each transcription "pulse" by a single RNA polymerase is composed of the succession of stages described in the two paragraphs above. In the absence of a transcription bubble,

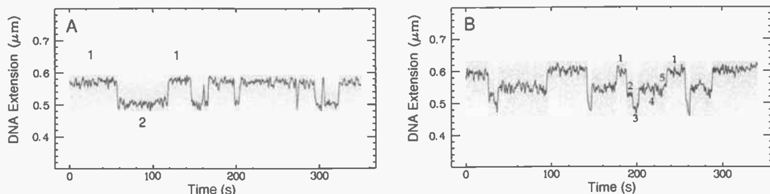

FIGURE 2 Real-time measurement of DNA extension allows for detection of RNA polymerase/DNA interaction. Raw data points are in light gray; the dark gray line corresponds to ~0.5-sec averaging. (*A*) In the absence of nucleotides, reversible promoter unwinding by a single RNA polymerase is observed as a transient *decrease* (state 2) in DNA extension. (*B*) RNA polymerase electrocardiogram: observation of the full transcription cycle by four successive RNA polymerase upon addition of all four nucleotides (ATP, UTP, GTP, and CTP). Labels for states 1–5 generated by the third of four RNA polymerases are as described in the text.

the DNA is in the state labeled *1*. Initial promoter unwinding is labeled as state *2*.

Initial promoter unwinding rapidly gives way to abortive cycling, heralded by a strong but transient expansion of the transcription bubble and labeled *3*. Abortive cycling appears to precede *all* promoter escape events and may thus be a requirement for promoter escape. During promoter escape, the transcription bubble rewinds noticeably, and the RNA polymerase then transcribes the ensuing DNA transcript (state *4*). Upon reaching a terminator sequence, the transcription bubble closes (state *5*) and the single-molecule time trace returns to baseline (state as in *1*). These experiments open many important new perspectives for understanding how these various stages of the transcription cycle all participate in the regulation of gene expression.

DNA Compaction by SMC Proteins

T. Kawaguchi, T. Hirano, T.R. Strick

The formation of compact mitotic chromosomes, as they are observed in human karyotypes, is mediated by the structural maintenance of chromosome (SMC) protein complex known as condensin. To better understand the vital process of chromosome condensation, we analyzed the response of nanomanipulated DNA to purified *Xenopus laevis* condensin.

Using DNA nanomanipulation, we were able to observe large-scale, rapid, and yet reversible condensation of the DNA upon addition of purified *X. laevis* condensin and ATP. This required both the phosphorylation patter consistent with the mitotic state of the cell cycle and access to hydrolyzable ATP. Control experiments performed in the absence of ATP show that under these conditions, the condensin complex binds reversibly to DNA but does not cause its condensation. At low protein concentration, the incremental compaction of DNA was observed as large (~90 nm) and discrete changes in DNA extension, and presumably corresponds to the association and dissociation of a single condensin complex from DNA. At higher condensin concentrations, when several condensins have bound to the nanomanipulated DNA, protein–protein interactions generate large-scale loops along the DNA. The formation of compact chromosomes would thus presumably be based on the formation of such loops along DNA.

Torque-induced Structural Transitions in DNA

T.R. Strick, R. Sachidanandam

These experiments aim to better understand the physical properties of DNA. We have observed that an unwinding torque applied to DNA induces abnormally slow and transient alterations of the molecule's secondary structure. Analysis of these conformational changes could help us understand how DNA supercoiling affects processes such as the initiation of DNA replication or the termination of transcription. In the last year, we showed that these fluctuations can be caused by DNA sequences containing imperfect inverted repeats, or palindromes. We are currently assessing the role of such fluctuations in the living cell.

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 (EBs). Previous studies have demonstrated that EBs 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 EBs.

At my bench, the initial goal of my 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 use 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 toehold for preclinical applications of stem cells in cell replacement therapies.

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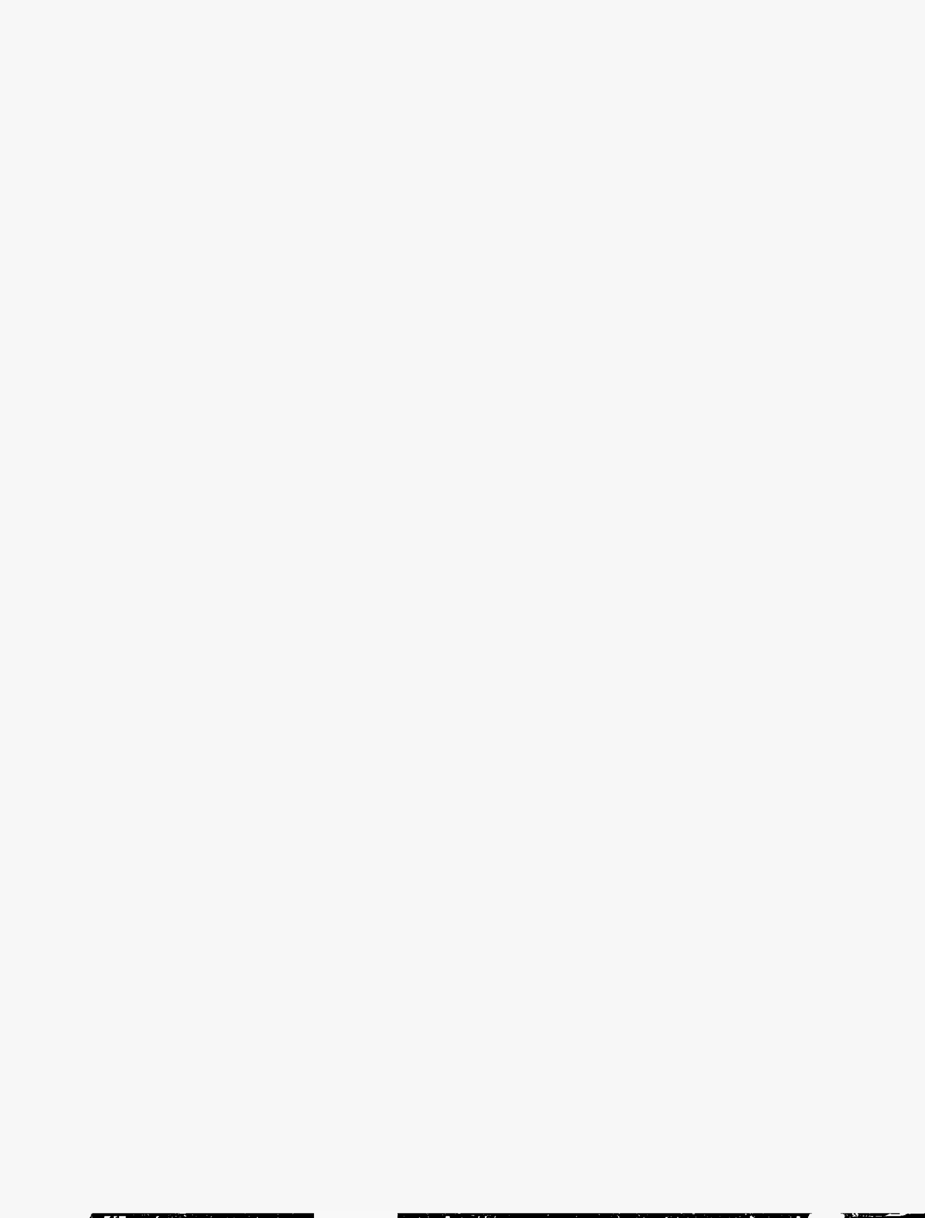

WATSON SCHOOL
OF BIOLOGICAL SCIENCES

Winship Herr, Ph.D., *Dean, Lita Annenberg Hazen Professor of Biological Sciences (January–July)*
Lilian Clark Gann, Ph.D., M.B.A., *Assistant Dean (January–June), Dean (July–)*
William Tansey. Ph.D., *Director of Graduate Studies, Lita Annenberg Hazen Professor of Biological Studies (July–)*
Alyson Kass-Eisler, Ph.D., *Postdoctoral Program Officer and Curriculum Administrator*
Dawn Meehan, B.A., *Admissions, Recruitment, and Student Affairs Manager*
Mark E. Beavers, B.S., *Administrative Assistant and Assistant to the Dean*

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Hollis Cline
Scott Lowe
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Lincoln Stein
Nicholas Tonks
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Catherine Y. Cormier, Watson School (January–February)
Marissa Moore, Stony Brook University (January–August)
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Mark Beavers (September–)
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Linda Van Aelst

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Professor, Department of Biology and Center for Cancer Research,
Massachusetts Institute of Technology

WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

This year saw two memorable events in the short history of the Watson School. First, on April 25, the efforts and dedication of our students and faculty came to fruition with the awarding of the School's first doctoral degrees. This was a tremendously gratifying and inspiring experience. Second was the retirement on July 1 of our founding dean, Winship Herr. The School and Cold Spring Harbor Laboratory (CSHL) will be forever indebted to Winship for his vision, leadership, and creativity, and for building a solid foundation from which the Watson School can continue to prosper.

First Students Graduate from the Watson School

On April 25, 2004, exactly 51 years after the publication of the double-helical structure of DNA by James Watson and Frances Crick, six Watson School students—Amy Caudy, Elizabeth Thomas, Niraj Tolia, and Emiliano Rial Verde from the first Entering Class of 1999, and Ira Hall and Patrick Paddison from the second Entering Class of 2000—received Ph.D. degrees conferred by Cold Spring Harbor Laboratory. The average time to degree for the six graduates was exactly four years! Also on that day, James Watson, Joan Steitz (a previous graduate student of Jim's), and Shirley Tilghman, president of Princeton University, all received honorary doctorates. It was a truly momentous occasion in the history of the Laboratory.

(Front row, left to right) Lilian Clark Gann, Patrick Paddison, Elizabeth Thomas, Amy Caudy, Emiliano Rial Verde, Winship Herr. *(Back row, left to right)* Ira Hall, Niraj Tolia, Bruce Stillman

The graduation was a wonderful affair. All six students spoke about their most treasured experiences at the Laboratory. Keith Yamamoto, the founding chair of the Watson School's External Advisory Committee, spoke about the courage the first pioneering students had to have in order to join this new school. He suggested that the first students were akin to students joining a new skydiving school where the instructors were teaching an untested skydiving method with which they had no prior experience of their own! Shirley Tilghman gave the keynote address and on a more serious note emphasized the need for continued government support of all the sciences. In her commencement speech, Dr. Tilghman had this to say about the Watson School:

> *You have shown that a graduate program can be accommodated successfully in four to five years without confining your intellectual thoughts to a small, narrow box. On the contrary, this school has taught you to think in broader terms than many graduate programs do—integrating instruction and research throughout your time here.*

The graduates were an impressive group indeed. Amy Caudy, Emiliano Rial Verde, and Elizabeth Thomas were all recipients of the esteemed Howard Hughes Medical Institute predoctoral fellowship;

THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 1999

Michelle L. Cilia, September 28, 2004

Mechanisms of Intercellular Communication in Arabidopsis thaliana.

Thesis Examining Committee
Chair: **David L. Spector**
Research Mentor: **David Jackson**
Academic Mentor: **Nouria Hernandez**
Committee Member: **Philip Benfey**
Committee Member: **Robert Martienssen**
External Examiner: **Richard Nelson,** Samuel Roberts Noble Foundation, Inc.

Emiliano M. Rial Verde, April 1, 2004

Role of the Immediate-Early Gene Arc In Synaptic Transmission.

Thesis Examining Committee
Chair: **Roberto Malinow**
Research Mentor: **Hollis Cline**
Academic Mentor: **Jan A. Witkowski**
Committee Member: **David Helfman**
Committee Member: **Paul Worley**
External Examiner: **Gail Mandel,** Stony Brook University

Elizabeth E. Thomas, April 2, 2004

Short, Local Duplications: A New Type of Genomic Change.

Thesis Examining Committee
Chair: **Lincoln Stein**
Research Mentor: **Michael Wigler**
Academic Mentor: **William Tansey**
Committee Member: **Dmitri Chklovskii**
Committee Member: **W. Richard McCombie**
External Examiner: **Paul Bingham,** Stony Brook University

Niraj H. Tolia, March 31, 2004

Structural and Biochemical Studies of the Malaria Parasite Plasmodium Falciparum Protein EBA-175 Involved in Erythrocyte Invasion.

Thesis Examining Committee
Chair: **Rui-Ming Xu**
Research Mentor: **Leemor Joshua-Tor**
Academic Mentor: **David Helfman**
Committee Member: **Yuri Lazebnik**
Committee Member: **Scott Lowe**
External Examiner: **Stephen A. Johnston,** University of Texas–Southwestern Medical Center

ENTERING CLASS OF 2000

Zachary B. Lippman, December 15, 2004

Transposons, Heterochromatin, and Epigenetic Landscapes in Arabidopsis thaliana.

Thesis Examining Committee
Chair: **David Jackson**
Research Mentor: **Robert Martienssen**
Academic Mentor: **William Tansey**
Committee Member: **W. Richard McCombie**
Committee Member: **Eric Richards**
External Examiner: **Michael Ashburner,** University of Cambridge, United Kingdom

Patrick J. Paddison, April 1, 2004

RNA Interference in Mammals: The Quest for Illuminating Gene Function.

Thesis Examining Committee
Chair: **Scott Lowe**
Research Mentor: **Gregory Hannon**
Academic Mentor: **Adrian R. Krainer**
Committee Member: **Senthil K. Muthuswamy**
Committee Member: **Michael Wigler**
External Examiner: **Stephen J. Elledge,** Harvard Medical School

Amy Caudy won the prestigious Harold M. Weintraub Graduate Student Award for being one of the top 15 doctoral students selected in an international competition by the Fred Hutchinson Cancer Research Center in 2003; and Ira Hall was co-winner of the Newcomb Cleveland Prize for being a co-author on each of two articles selected as the best to be published by *Science* magazine in 2002. Some of the students (Amy Caudy and Elizabeth Thomas) have gone off to pursue postdoctoral experiences elsewhere, others (Ira Hall and Patrick Paddison) are Cold Spring Harbor Fellows, and the other two (Emiliano Rial Verde and Niraj Tolia) are continuing postdoctoral studies at the Laboratory. We wish them all every success!

Winship Herr

Founding Dean Winship Herr Steps Down

Winship Herr stepped down as dean of the Watson School of Biological Sciences at Cold Spring Harbor Laboratory on July 1, 2004—having dedicated the better part of a decade to the establishment of our successful, innovative Ph.D. program—to concentrate on his research.

Beginning in 1995, Winship spearheaded an effort that resulted, in September 1998, in the Laboratory's accreditation as a Ph.D. degree-granting institution by the Board of Regents of the University of the State of New York, on behalf of the State Education Department. And so the Watson School became a reality. Soon thereafter, Winship became founding dean of the Watson School. Since its founding, we have seen the School grow and become one of the most innovative programs in the country, attracting outstanding students. Winship nurtured and shaped the Watson School

Winship Herr with students at their first lecture using 3D molecular graphics projection.

Winship Herr with founding instructors.

in its first formative years, culminating in the first Commencement Convocation this spring. We all thank Winship for all his outstanding efforts on the School's behalf. He has helped shape the future of Cold Spring Harbor Laboratory in a unique way.

In addition to being founding dean, Winship's roles at the School were many and varied, including serving on the School's Executive Committee, Admissions Committee, and Qualifying Exam Committee. He was an instructor on the Scientific Reasoning and Logic core course and the *Mechanisms of Transcriptional Regulation: From* E. coli *to Elephants* specialized disciplines course, as well as a guest instructor for the Scientific Exposition and Ethics core course. If that was not enough, Winship was also a laboratory rotation advisor as well as a thesis research advisor. Winship has certainly left us with very big shoes to fill!

I was deeply honored that the Laboratory appointed me as Winship's successor as dean. I joined the Laboratory in March 1999 as assistant dean of the School and was promoted to associate dean in January 2002. On July 1, Dr. William Tansey joined me at the "helm" of the Watson School. Bill was appointed director of graduate studies and the Lita Annenberg Hazen Professor of Biological Sciences. We are very excited to be given this opportunity to lead the School and to build on its initial successes.

The Fall Term Curriculum Is Revamped

The Curriculum Development and Integration Committee, comprising Bill Tansey (chair), myself, David Spector, and Nicholas Tonks, continues to carefully monitor and develop the curriculum. For fall 2004, the Scientific Reasoning and Logic (SRL) core course underwent a significant reorganization, although the content of many of the modules remained the same. Winship Herr and Leemor Joshua-Tor stepped down as full instructors, and Carlos Brody and Marja Timmermans were recruited to fill these spots. The course is now divided into six modules: Gene Expression, Signal Transduction, Cell Division Cycle, Development, Neuroscience, and Study Section. The total number of lectures and discussions did not change, and many of the lectures themselves remained the same. Importantly, there was only one (standard length) problem set per module, thus reducing by approximately half the amount of material students needed to write and submit for this course, giving them more time to think about the topic and the problem sets. The Study Section module—in which students participated in a panel review of National Institutes of Health R01 grant applications—was entirely new. The logic behind this module is that the skill of understanding published work is different from the skill of analyzing science that is not yet done—evaluating the questions before the answers are known, evaluating routes toward discovery before knowing where they will end, and making critical judgments about how to proceed in the face of an uncertain future.

The Scientific Exposition and Ethics (SEE) core course was also restructured considerably, although several of the weekly lectures remained the same. This restructuring was motivated by a number of factors. Adrian Krainer, who was one of the founding instructors of the SEE course, decided not to teach in 2004. Yuri Lazebnik was recruited as his replacement. The instructors also wanted to include a significant, multiweek, assignment that focused on grant-writing skills, and this was difficult to achieve with the old course format. The grant-writing exercise is meant to complement the SRL Study Section module, and its primary purpose is to guide students through the preparation of a grant proposal (which, in reality, is very close to a Watson School thesis proposal). The topic for the grant proposal was *Regulation of Transcription by Gal4*. This topic was selected because it was a common theme both in the SRL transcription lectures and in the *Molecules to Networks* specialized disciplines course; material from both of these courses was used as the basis for the grant application. It is hoped that this restructuring of the SEE course will provide a better format to develop students' writing skills.

The Curriculum Development and Integration Committee interacts closely with potential specialized disciplines course instructors not only to identify topics, but also to help them develop courses and their methods of assessment, and to monitor workloads. For fall 2004, the *Systems Neuroscience* course was replaced by *Molecules to Networks* (a proteomics course), taught by W. Richard McCombie (lead instructor), Leemor Joshua-Tor, and Michael Myers.

ENTERING CLASS OF 2004

Daniel H. Chitwood, University of California, Davis
George A. and Marjorie H. Anderson Fellow
National Science Foundation Graduate Research Fellow

Galen A. Collins, Wabash College
Beckman Graduate Student

Oliver I. Fregoso, University of California, Santa Cruz
William Randolph Hearst Scholar
Seraph Cancer Research Scholar

Keisha A. John, University of Maryland, Baltimore County
William Randolph Hearst Scholar
Ford Foundation Fellow

Shraddha S. Pai, University of Waterloo
Charles A. Dana Fellow

Molly R. Perkins, Harvard University
Cashin Fellow

Evan E. Santo, Rochester Institute of Technology
Robert and Teresa Lindsay Fellow

David R. Simpson, University of California, Davis
Beckman Graduate Student

Entering Class of 2004

On August 30, 2004, the Watson School opened its doors for the sixth time to welcome yet another new class. Eight students—Daniel Chitwood, Galen Collins, Oliver Fregoso, Keisha John, Shraddha Pai, Molly Perkins, Evan Santo, and David Simpson—comprise the entering class of 2004. Daniel Chitwood and Keisha John are the recipients of a National Science Foundation predoctoral fellowship, and a Ford Foundation predoctoral fellowship, respectively.

(*Left to right*) Daniel Chitwood, David Simpson, Galen Collins, Molly Perkins, Keisha John, Evan Santo, Oliver Fregoso, Shraddha Pai

Recruiting Efforts

Recruitment of the graduate program's entering class of 2005 and of participants for our summer 2005 Undergraduate Research Program (URP) will be managed by Dawn Meehan, the School's admissions, recruitment, and student affairs manager. Throughout 2004, Dawn traveled the length and breadth of the country to recruit students to our graduate and undergraduate programs. She has done an outstanding job at representing Cold Spring Harbor Laboratory and the Watson School and I am deeply indebted to her for her efforts. Dawn, assisted at times by faculty, students, and members of the School's administration, attended recruitment fairs and conferences in California, Maryland, Massachusetts, New York, Texas, and Washington D.C.

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 2004 entering class comprised Marja Timmermans (chair), Carlos Brody, Gregory Hannon, Adrian Krainer, Alea Mills, Michael Myers, Andrew Neuwald, Karel Svoboda, and Linda Van Aelst. They are a truly remarkable team!

DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
ENTERING CLASS OF 2000			
Santanu Chakraborty *George A. and Marjorie H. Anderson Fellow*	Michael Wigler	Carlos D. Brody	Mechanisms of robust short-term memory in biological networks
Elena S. Ezhkova *Engelhorn Scholar*	Jan A. Witkowski	William Tansey	Role of the proteasome in transcription
Rebecca C. Ewald *Engelhorn Scholar*	Bruce Stillman	Hollis Cline	NMDA receptor trafficking and its impact on neuronal functionality and morphology
Zachary Bela Lippman *Beckman Graduate Student* **Thesis Defense: Dec. 2004**	William Tansey	Robert Martienssen	Comprehensive analysis of chromatin status on *Arabidopsis* chromosome 4
Marco Mangone *Charles A. Dana Foundation Fellow*	Linda Van Aelst	Winship Herr	Role of HCF-1 in cell proliferation
Masafumi Muratani *George A. and Marjorie H. Anderson Fellow*	Nouria Hernandez	William Tansey	Gene regulation by ubiquitin-mediated proteolysis
Patrick J. Paddison *Beckman Graduate Student* **Thesis Defense: April 2004**	Adrian R. Krainer	Gregory Hannon	An RNAi-based screen in mouse embryo fibroblasts for transformation-lethal gene targets
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	Mapping the trafficking of AMPA receptors in dendritic compartments
Ji-Joon Song *Bristol-Myers Squibb Fellow*	Scott Lowe	Leemor Joshua-Tor	Structural studies of RNAi
Dougal G.R. (Gowan) Tervo *George A. and Marjorie H. Anderson Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Carlos D. Brody	Karel Svoboda	An inducible and reversible lesion of the corticothalmic projection

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 mixing Stony Brook University and Watson School students. The graduate student seminars, which are held each September through May, allow 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 five faculty mentors whose attendance rotates each week. The seminar series serves three important roles: (1) It gives students an opportunity to hone their oral presentation skills; (2) it enables students to defend their research in the absence of their research mentors, such that they experience standing on their own two feet as they will have to throughout their careers; and (3) it allows the students in the audience to ask questions within their own peer group, thus learning the important roles

DOCTORAL THESIS RESEARCH (continued)

Student	Academic Mentor	Research Mentor	Thesis Research
ENTERING CLASS OF 2002			
Allison L. Blum *Barbara McClintock Fellow*	Leemor Joshua-Tor	Josh Dubnau	Genetic, behavioral, and anatomical characterization of Radish-dependent memory
Darren Burgess *Engelhorn Scholar*	Nicholas Tonks	Scott Lowe	Mammalian RNAi genetic screens: Discovery and characterization of genes mediating the response to cancer therapy
Beth L. Chen *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 receptors 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 *Elisabeth Sloan Livingston Fellow*	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 A. Witkowski		To be determined
Christopher Harvey *David and Fanny Luke Fellow*	Adrian R. 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 *Leslie C. Quick Jr. Fellow*	Jan A. Witkowski	Roberto Malinow	Activity-dependent modulation of APP processing and Aβ production in rat hippocampal neurons

of audience participation for the advancement of research. At the end of the evening, the two faculty mentors give each presenting student a critique of the seminar. In addition, members of the audience complete (anonymously) seminar evaluation forms, which are then given to the presenting students.

Josh Dubnau, Terri Grodzicker, Alea Mills, and Arne Stenlund continue to serve as faculty mentors, and they were joined this year by Rui-Ming Xu who brings expertise in the areas of biophysics and structural biology.

Academic Mentoring

The Watson School takes great pride in the level of mentoring it offers to its students. One of the very special aspects in this regard is its academic mentoring program, led by Bill 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 the student through the sometimes trying process of a doctoral education. This program continues to receive much support from the faculty who volunteer to be academic mentors, and it has rightfully become a vital ingredient in the Watson School's success. This year's new academic mentors for the entering class of 2004 are:

Alea A. Mills (Daniel Chitwood)
Marja Timmermans (Galen Collins)
Adrian R. Krainer (Oliver Fregoso)
Bill Tansey (Keisha John)
Anthony Zador (Shraddha Pai)
John R. Inglis (Molly Perkins)
Vivek Mittal (Evan Santo)
Scott Lowe (David Simpson)

Interinstitutional Academic Interactions

It is important to always bear in mind that most of the graduate students studying at Cold Spring Harbor Laboratory are not Watson School students. Indeed, the largest percentage of students come from Stony Brook University. The Watson School provides an on-site "home" for all of these students to help them feel that they are a 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 below joined us.

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS

Student	CSHL Research Mentor	Program
Magda Gierszewska	Jacek Skowronski	Medical University (Gdansk), Biotechnology
Teresa Haire	Senthil K. Muthuswamy	Stony Brook, Microbiology
Aftabul Haque	Nicholas Tonks	Stony Brook, Molecular & Cell Biology
Katarzyna Hrecka	Jacek Skowronski	Medical University (Gdansk), Biotechnology
Justyna Janas	Linda Van Aelst	Medical University (Gdansk), Biotechnology
Noriko Kobayashi	Masaaki Hamaguchi	University of Tokyo, Virology/Genetics
Lukasz Kozaczkiewicz	Jacek Skowronski	International Max-Planck, Molecular Biology
Guang Lin	Nicholas Tonks	Stony Brook, Molecular and Cell Biology
Daniel J. Nolan	Vivek Mittal	Stony Brook, Genetics
Jasmin Roohi	Eli Hatchwell	Stony Brook, Genetics
Rahul Sinha	Adrian R. Krainer	Stony Brook, Molecular and Cell Biology
Francisca von Saint Paul	Karel Svoboda	International Max-Planck, Neuroscience
Seugtai Yoon	Michael Wigler	Stony Brook, Statistics
Chaolin Zhang	Michael Q. Zhang	Stony Brook, Biomedical Engineering
Zhen Zhao	Scott Lowe	Stony Brook, Genetics

Faculty Changes

One new research faculty member joined the Watson School this year. Scott Powers joined us as an associate professor and director of the Laboratory's Human Cancer Genome Center. Scott came to the Watson School from Tularik Genomics Division, which used RDA technology to identify cancer genes. At the Laboratory, his research will focus on utilizing ROMA technology to identify cancer genes with an emphasis on functional validation. We are excited to have him here and look forward to his participation in Watson School activities.

We also saw this year the departure of David Helfman who had been an integral part of the School since its inception. David's laboratory focused on studying the expression, structure, and function of cytoskeletal components in normal and transformed cells. Like Winship, David played a much-valued role in the Watson School. He served on the School's Executive Committee and was a faculty mentor (and the organizer) for the Graduate Student Seminar Series. He was an instructor on the Scientific Reasoning and Logic core course and the *Cellular Structure and Function* specialized disciplines course. David also served on two Watson School students thesis committees and was an academic mentor for the School. We are deeply indebted to him for all of his efforts and wish him well in his "new home" in the Department of Cell Biology and Anatomy at the University of Miami School of Medicine.

Graduate Students and Postdoctoral Fellow Departures

With each year comes not only new arrivals, but also new departures. The following graduate students and postdoctoral fellows departed from the Laboratory:

Graduate Students

Nilanjana Banerjee	Michelle L. Cilia	Chrostoph Grunwald
Jeong-Gu Kang	Sungwoo Lee	Arlinda Lee
Michelle T. Juarez	Manpreet Katari	Patrick J. Paddison
Anca Radulescu	Yvette R. Seger	Elizabeth E. Thomas
Niraj H. Tolia	Andriy Tovkach	Emiliano Rial Verde

Postdoctoral Fellows

Carlos Aizenman	Colin Akerman	Mary Byrne
Anna Lena Chabes	Wim Karel Deleu	Eric Drier
Jian Du	James Egan	Viola Ellison
Francois Grenier	Sunita Gupta	Kurt Haas
Andreas Herbst	Ping Hu	Ashkan Javaherian
Sibel Kantarci	Catherine Kidner	Yingfang Liu
Ana Losada	Juan Mendez	Marta Moita
Naoki Nakaya	Sarah Newey	Pablo Rabinowicz
Edward Ruthazer	Andrey Shabes	Ryushi Shudo
Sen Song	Armen Stepanyants	Jinhua Wang
Joseph West	Xuemei Zhao	

The Undergraduate Research Program

The summer Undergraduate Research Program (URP) has come under the umbrella of the Watson School since 1999, but its administration was handled elsewhere at Cold Spring Harbor Laboratory. Jane Reader, the scientific administrator of James Laboratory who had been the URP administrator for 15 years (and did an outstanding job!), relocated with her family to Massachusetts in June 2004. The

Watson School, and in particular Dawn Meehan, took over the administration of the URP, resulting in a somewhat more hectic summer than usual. But this also had its pluses. We were able to gain first-hand knowledge of the workings of the program and as such will be able to streamline for the future. The URP is proving to be a great source of graduate students for the Watson School. Fully 25% of our students have come via this program, including Galen Collins, Keisha John, and Shraddha Pai of the entering class of 2004.

The Postdoctoral Program at Cold Spring Harbor Laboratory

Now in its second year, 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 management of Alyson Kass-Eisler. This year, the Laboratory welcomed 42 new postdocs and saw 32 depart. The departing postdocs went on to positions at Brown University, Eli Lilly, Indiana University, The John Innes Centre, Merck Research Laboratories, Northeastern University, Oxford University, The Institute for Genomic Research, the Spanish National Cancer Institute, the University of British Columbia, and the University of Edinburgh, to name but a few. We wish them every success for the future.

Our second postdoc working group convened in October 2003 and was tasked with recommending to the Laboratory's President, Bruce Stillman, the structure of a comprehensive postdoctoral training program. Membership of the group included Winship Herr (chair); myself; Alyson Kass-Eisler (secretary); faculty members Leemor Joshua-Tor, Adrian R. Krainer, Senthil K. Muthuswamy, and Nicholas Tonks; and postdocs Kurt Haas, Michael Hemann, Sarah Newey, Vatsala Thirumalai, and Michael Wehr (who took over for Kurt when he left in January to take up a faculty position at the University of British Columbia). This group met seven times, concluding on June 6, 2004. Alyson has been busy synthesizing the wealth of ideas from these discussions and will shortly submit the group's recommendations to Bruce.

To complement our local efforts, we have been involved in workshops at a national level aimed at shaping the postdoctoral experience. In 2004, we became founding members of the National Postdoc Association (NPA). The NPA was founded by a dynamic group of postdocs from around the country who attended the 2002 National Postdoc Network meeting. A Laboratory representative has attended each of the National Postdoc Network and NPA meetings since 2001. Alyson Kass-Eisler, our Postdoctoral Program Officer, attended The National Academies Committee on Science, Engineering, and Public Policy, 2nd Convocation on "Enhancing the Postdoctoral Experience." At this meeting of leaders interested in improving the postdoctoral experience, Alyson presented a poster highlighting the postdoctoral experience at CSHL. Her presentation was well received by postdocs, administrators, faculty, and others in attendance.

Assisting postdocs with career development continues to be an important goal of CSHL. The Laboratory's 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 seminar titles included:

- From IDEA to IPO: The Technology Venture Course
- Bioscience Research: "Where the Jobs Are"—A Career Fair and Symposium
- Women in Science: Turning Choices into Success
- YOU KNOW YOUR SCIENCE, BUT CAN YOU TEACH IT?: The Top Ten Things That Successful College Professors Do

A new workshop on the *Responsible Conduct of Research* was developed this year for postdocs at the Laboratory. This two-part workshop includes instruction in the use of animals and humans in research, biosafety, scientific publishing and funding, instruction on data handling, scientific misconduct, patents and intellectual property, and scientific communication. The first of the two workshops

took place on August 25. Instructors included Diane Esposito, a research investigator and chair of the Laboratory's Institutional Review Board, who discussed "Protecting Human Subjects" and also provided an historical overview of the use of human subjects in research. Jan Gnadt, DVM, the Lab's veterinary consultant, and Lisa Bianco, manager of Animal Resources, gave a presentation on "Ethics and Animal-based Research," which covered the humane and ethical treatment of animals, USDA and Public Health Service animal welfare regulations, and the use of nonanimal alternatives in research. John Pisciotta, the Laboratory's manager of Environmental Health and Safety, talked about "Biosafety in the Research Laboratory." He described the National Institutes of Health guidelines for the use of recombinant DNA and the more recent regulations regarding the use of select agents, those identified by the Centers for Disease Control as posing a risk for use as biological weapons.

This year, with the help of CSHL's Department of Media Arts and Visualization, we launched a much-improved Postdoctoral Research Web site. Our site now has a listing of distinguished alumni, links to faculty research expertise, a description of the Laboratory's National Cancer Institute (NCI) training grant and NCI Cancer Center resources, and links to Laboratory benefits for postdocs. The CSHL Postdoctoral Association (PDA) continues to hold meetings every three months or so to discuss life as a postdoc at CSHL. These meetings are open to all postdocs at the Laboratory. One area the PDA has been hard at work on is developing their own Web site, with a view to providing practical information on being a CSHL postdoc, which will no doubt be a valuable resource for current and future postdocs at the Laboratory. In addition, Alyson Kass-Eisler continues to act as a contact and provider of information and resources for postdocs locating to, or thinking of locating to, the Laboratory. She is extremely well placed to do this, having been a CSHL postdoctoral fellow herself!

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. The year saw several major changes in the membership of the Committee, due in large part to Winship Herr stepping down as dean.

January 2004 saw the induction of two new faculty members—W. Richard McCombie and Nicholas Tonks. In July, Winship Herr retired and I was appointed chair of the Executive Committee with Bill Tansey as vice chair. Alyson Kass-Eisler took over from Mark Beavers in September as committee secretary, and as happens each year, there was also turnover among the student representatives. The Watson School representative Catherine Cormier was replaced by Darren Burgess and the Stony Brook University representative Marissa Moore was replaced by Marlies Rossmann. The School is indeed thankful for their frank, honest, and thoughtful advice.

External Advisory Committee

Since its inception in 1998, the Watson School has relied heavily on advice from the External Advisory Committee (EAC). With the program now more than five years old, I was most pleased that the EAC, led by Keith Yamamoto, was able to come in September for a site visit. The site visit focused on five main areas: (1) faculty, (2) students, (3) curriculum, (4) a review and views on the first five years of the Watson School, and (5) the next five years and beyond. As usual, the team met with students, faculty, and administrators, and the overall feeling was that our "grand experiment" was indeed working. At the site visit, we were presented with the findings of their previous visit where they had this to say about our faculty: "Their dedication and tenacity have been inspiring ... What is remarkable and impressive is the faculty's stamina in remaining focused on the many responsibilities inherent in creating an outstanding program."

The EAC's continued commitment to the success of the Watson School has been a humbling experience—Thank you so much guys!

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, which is dedicated to the graduate students at the Laboratory. Richard Losick from Harvard University was this year's Fellow. He was a particularly appropriate choice given that he is co-author on the recently published fifth edition of James Watson's *Molecular Biology of the Gene*. In addition to his lecture on his studies of development in the sporulating bacterium *Bacillus sublilus*, Rich spoke at a roundtable with the students about the life of a scientist.

Changes in the Watson School

With the departure of founding dean Herr came the opportunity to review the administration and oversight of the Watson School and its programs, which currently include:

- The WSBS graduate program
- All other graduate programs at CSHL, mainly students from our shared programs with Stony Brook University
- The CSHL postdoctoral program
- The Undergraduate Research Program
- The Partners for the Future (PFF) Program for high school students
- The Nature Study Program, a summer program for elementary and secondary school students

(*Left to right*) Mark Beavers, Dawn Meehan, Lilian Clark Gann, Alyson Kass-Eisler, Bill Tansey

I was appointed dean on July 1, 2004 and, by virtue of office, also became one of the Laboratory's seven division directors. Bill Tansey was appointed director of graduate studies and Lita Annenberg Hazen Professor of Biological Sciences. Upon assuming my appointment as dean, I carried out a review of the administration of the School and decided at that time not to make too many changes, but rather to reshuffle and expand the roles and responsibilities of the existing "Team Watson." Dawn Meehan's promotion to admissions, recruitment, and student affairs manager relieved her of curriculum administration. To fill this gap, Alyson Kass-Eisler stepped up to the plate; she switched from working three days a week to full time. She is still the Postdoc Program Officer three days a week—on the other two days, she administers the Watson School graduate program curriculum under the direction of Bill Tansey. Mark Beavers continues to provide outstanding administrative support to all at the School as well as fulfilling the role of assistant to the dean. We are indeed very fortunate to have such a dedicated and hardworking team—I cannot thank them enough for all of their efforts.

The Watson School Continues to Benefit from Generous Benefactors

The Watson School has been extremely fortunate to be the beneficiary of many generous benefactors who have provided grants and endowed funds for student, faculty, and administrative support.

This fall, I was most pleased to host Jacqueline Dorrance, Executive Director of the Arnold and Mabel Beckman Foundation, for a site visit to CSHL, with the help of our chief development officer,

Charles Prizzi. During the visit, Ms. Dorrance had an opportunity to meet with faculty members Hollis Cline, Gregory Hannon, Winship Herr, and Bill Tansey; Laboratory President Bruce Stillman; Chancellor James Watson; and of course the students. Watson School students Beth Chen, Catherine Cormier, Ira Hall, Zachary Lippman, Patrick Paddison, and Izabela Sujka, who are all supported by the Arnold and Mabel Beckman Foundation, participated in a mini-symposium for Ms. Dorrance during which they described their thesis research. The students were very impressive and all greatly enjoyed the occasion.

Dean Gann Appointed to the Regents Advisory Council

A major milestone for the Watson School is its involvement in reviewing the academic credentials of other New York State educational institutions. Having completed my training as a peer reviewer for institutional accreditation for the New York State Education Department, I was invited to participate in an accreditation site visit of the Graduate College of Union University in Schenectady in May. I chaired the site visit team and found the experience invaluable as I learned what the State Education Department and its reviewers look for when evaluating doctoral programs. In addition, in November, I was appointed to the New York State Board of Regents Advisory Council for Institutional Accreditation for an initial term of three years.

In closing, I would also like to share with you some of the nonacademic highlights the Watson School has seen this year. In May, we saw the birth of Ji-Joon Song's (entering class of 2001) second child Juho Nathan. Michelle Cilia (entering class of 1999) married H. Kern Reeve in November. And in December, Darren Burgess (entering class of 2003) became engaged to Fiona Holloway. Our congratulations and best wishes to all of them!

Lilian Clark Gann
Dean

SPRING CURRICULUM

TOPICS IN BIOLOGY

ARRANGED BY **Dawn Meehan**
Jan A. Witkowski

FUNDED IN PART BY **The Daniel E. Koshland, Jr. Visiting Lectureship; The David Pall Visiting Lectureship; The Fairchild Martindale Visiting Lectureship; The Lucy and Mark Ptashne Visiting Lectureship; The Michel David-Weill Visiting Lectureship**

Each year, one or a team of invited instructors offer seven-day courses at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the Cold Spring Harbor Laboratory Lecture Courses held each summer at the Banbury Conference Center. In spring 2004, there were two such courses: Immunology and Microbial Pathogenesis.

Immunology

Attended by the entering classes of 2000 and 2003

INSTRUCTOR **Hidde Ploegh**, Harvard Medical School

TEACHING FELLOWS **Marianne Boes**, Harvard Medical School
Andre Catic, Harvard Medical School
Silke Paust, Harvard Medical School

This course introduced the elements of the immune system that make it a unique object of biological studies, with cross references to some of the more applied aspects. Its challenge was to convey a

(*Left to right*) Marianne Boes, Santanu Chakraborty, Despina Siolas, Masafumi Muratani, Silke Paust, Izabela A. Sujka, Hidde Ploegh, Angélique Girard, Christopher D. Harvey, Wei Wei, François Bolduc, Hiroki Asari, Rebecca A. Bish, Rebecca C. Ewald, Andre Catic, Khalid Siddiqui, Marco Mangone

sense of what the immune system is about. The course ran from Sunday to Saturday, February 29–March 6, and was organized and taught by Hidde Ploegh. Three teaching fellows participated in all aspects of the course. As in previous years, the course was highly rated by all of the students.

Microbial Pathogenesis

Attended by the entering classes of 2001 and 2002

INSTRUCTORS
Stanley Maloy, San Diego State University
Ronald K. Taylor, Dartmouth Medical School

GUEST LECTURERS
Andrew Darwin, New York University School of Medicine
Heran Darwin, Weill Medical College of Cornell University
Darren Higgins, Harvard Medical School
Theresa Koehler, University of Texas, Houston Medical School
Paula Sundstrom, Dartmouth Medical School

This course focused on mechanisms of microbial pathogenesis and the host response, and the scientific approaches that are used to investigate these mechanisms. How do microbes adhere to host cells? How do environmental cues direct the response of microbial pathogens? How do microbial pathogens modulate host cells to expedite virulence? How do host cells respond to microbial pathogens? How does the host immune system react to microbial pathogens? What does genomics tells us about how microbial pathogens evolve? How do emerging pathogens take advantage of new ecological niches? Although there are numerous microbial pathogens, the answers to these questions indicate that many pathogens use similar approaches to solve common problems.

The course ran from Sunday to Saturday, May 2–9, and was organized and largely taught by Stanley Maloy and Ronald Taylor. It integrated lectures by the instructors, daily quizzes, directed readings of research papers, and seminars by the instructors plus the five invited guest lecturers who specialize in various aspects of bacterial pathogenesis. The students, many of whom had little prior awareness of the subject, rated the course very highly.

(*Top row, left to right*) Ronald K. Taylor, Darren Higgins, Beth L. Chen, Shu-Ling Chiu, Stanley Maloy, Claudia E. Feierstein, Elizabeth Murchison, Jonathan Kui, Dougal G.R. (Gowan) Tervo, Darren Burgess. (*Bottom row, left to right*) Ji-Joon Song, Tomás Hromádka, Catherine Y. Cormier, Allison L. Blum, Charles D. Kopec

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Scott Bronson**
Uwe Hilgert
Craig Hinkley
Kimberly Kessler
Erin Maroney
Amanda McBrien
Mike O'Brien

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 experiences.

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 with the opportunity to get to know one another and to explore possibilities for doctoral thesis research. At the end of each rotation, students make short presentations of their studies to the other students and their rotation advisors. These talks give students an opportunity to share their laboratory experiences and to learn how to give a scientific presentation. With this latter goal in mind, in addition to the research mentors, the instructors of the Scientific Exposition and Ethics core course attend the talks and give individual feedback to students on their presentations. This year, 16 faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS

Hollis Cline
Gregory Hannon
Z. Josh Huang
Scott Lowe
Roberto Malinow
Alea A. Mills
Partha P. Mitra
Senthil K. Muthuswamy
Michael P. Myers
Karel Svoboda
William Tansey
Marja Timmermans
Tim Tully
Linda Van Aelst
Jan A. Witkowski
Anthony Zador

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

INSTRUCTORS
Gregory Hannon (Lead)
Carlos D. Brody
Hollis Cline
Senthil K. Muthuswamy
Arne Stenlund
Marja Timmermans

GUEST LECTURERS
Grigori Enikolopov
Z. Josh Huang
David Jackson
Adrian R. Krainer
Wolfgang Lukowitz
Zachary Mainen
Patrick J. Paddison
Cordula Schulz
Bruce Stillman
William Tansey

VISITING LECTURERS
Howard Crawford, Stony Brook University
William C. Merrick, Case Western Reserve University
Howard Sirotkin, Stony Brook 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 the 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	Neuroscience
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

GUEST LECTURERS **Terri Grodzicker**
Adrian R. Krainer

VISITING LECTURERS **Robert P. Charrow, Esq.,** Greenberg Traurig LLP
Jasemine Chambers, United States Patent and Trade Office
Peter Petre, Fortune Magazine
Philip Reilly, J.D., M.D., CEO of Interleukin Genetics
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 **Mark Beavers**
Lilian Clark Gann

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. Weekly speakers were:

Week 1 Andrew Neuwald, Rui-Ming Xu
Week 2 Scott Lowe, Linda Van Aelst, David L. Spector
Week 3 Alea A. Mills, Senthil K. Muthuswamy
Week 4 William Tansey, Michael Wigler
Week 5 Robert Martienssen, W. Richard McCombie
Week 6 Tatsuya Hirano, Bruce Stillman, Arne Stenlund
Week 7 Josh Dubnau, Robert Lucito, Karel Svoboda
Week 8 Dmitri Chklovskii, Zachary Mainen, Tim Tully

Week 9	David Jackson, Wolfgang Lukowitz, Marja Timmermans
Week 10	Masaaki Hamaguchi, Eli Hatchwell, Vivek Mittal
Week 11	Carlos D. Brody, Hollis Cline, Anthony Zador
Week 12	Grigori Enikolopov, Z. Josh Huang, Leemor Joshua-Tor
Week 13	Alexei Koulakov, Lincoln Stein, Michael Q. Zhang
Week 14	Gregory Hannon, Partha P. Mitra, Yi Zhong
Week 15	Adrian R. Krainer, Michael P. Myers

SPECIALIZED DISCIPLINES COURSES

Molecules to Networks

FUNDED IN PART BY **The George W. Cutting Lectureship; The Klingenstein Lectureship; The William Stamps Farrish Lectureship**

INSTRUCTORS
W. Richard McCombie (Lead)
Leemor Joshua-Tor
Michael P. Myers

VISITING LECTURERS
Brian Chait, The Rockefeller University
Michael Tyers, University of Toronto
Marc Vidal, Harvard University

The history of molecular biology has been focused on the reduction of biology to the basic building blocks of the cell. Recent advances in genomics, proteomics, and structural biology open the possibility of moving in the other direction, toward an integrated view of how the cell functions. We can now take the knowledge we have of simple circuits, interactions, and protein structure and study how these elements combine into the complex networks that regulate cellular processes. This course focused on the macromolecular interactions that are used to regulate gene expression, from the simplest protein–protein interface to higher-order protein networks that coordinate patterns of gene activity. It began with analysis of a basic genetic regulatory circuit, a discussion of the fundamentals of protein structure, and a description of protein–protein interactions. It then expanded the discussion to reveal how these basic principals are elaborated and repeated, with slight variation, to form complex networks in the cell. Simple bacterial systems were used to demonstrate several basic themes, but the majority of the course highlighted the galactose gene regulation system of yeast, which has been extensively studied at both the reductionist and holistic levels. At the conclusion of the course, students were expected to understand not only the basics of gene regulation, structure, and protein analysis, but also how elaborate protein networks evolve and operate.

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**
GUEST LECTURER	**Tatsuya Hirano**
VISITING LECTURERS	**Michael Caplan**, Yale University **Gregg Gundersen**, Columbia University **Marc Symons**, Picower Institute **Graham Warren**, Yale University

This course highlighted the use of various molecular and biochemical approaches to address problems in cell biology. The overall goal was to give students an idea as to how different approaches are used and integrated to address specific research questions. We discussed how molecular biology could be used to dissect various problems in cell biology, including elucidating the primary structure of a protein, preparation of recombinant proteins, introduction of wild-type and mutant proteins into cells, structure-function studies, and targeted disruptions. Using specific examples, we introduced the students to the use of biochemistry in understanding problems in cell biology.

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**
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 were integrated "vertically" to understand how causal models of gene function are inferred across various levels of biological organization and lead to molecular mechanisms. Data were also integrated "horizontally" to understand how genetic pathways have been conserved evolutionarily as variations on a theme.

This course placed classic organismal genetics into the context of modern molecular biology and genomics. History, perspective, and technique were described around four levels of analysis: phenotype, genotype, variation, and genome. How do gene mutations disrupt phenotypic processes? How are complex traits genetically dissected into functional components? What concepts and techniques are used to organize genes into pathways and networks? How are genes mapped, cloned, and engineered to identify functional domains of proteins? What gene variation exists in natural populations? What are the functional consequences of gene variation? How 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.

UNDERGRADUATE RESEARCH PROGRAM

Program Director: Senthil K. Muthuswamy

Program Administrator: Jane Reader (January–June)
Dawn Meehan (June–)

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, 651 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, bioinformatics, and genomics; and (4) a familiarization 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 448 applicants, took part in the 2004 program:

(*Front row, left to right*) Johanna Berberena, Heeran Buhecha, Miranda Kim, Srinjan Basu, Beatrice Tapawan. (*Second row, left to right*) Pawel Mazur, Emily Heikamp, Bao Pham, Carol Cho. (*Third row, left to right*) Amy Leung, Max Jan, Jakob Macke, Siddharth Srivastava, Boaz Gildor, Nicholas Wall, John Calarco, Juan Aragon, Katrina Gold, Carissa Meyer, Matthew Klein, Brianna Burden, Eric Sullivan.

Juan Aragon, Armstrong Atlantic State University
Advisor: **Gregory Hannon**
Sponsor: Burroughs Wellcome
Mapping of the interaction of the 5′ end of siRNA with Argonaute.

Srinjan Basu, Cambridge University
Advisor: **Senthil K. Muthuswamy**
Sponsor: Shakespeare Fund
Role of *par* genes in cell proliferation.

Johanna Berberena, Hunter College
Advisor: **Josh Dubnau**
Sponsor: Garfield Fund
Expression study of long-term memory gene *Thor* (4E-BP).

Heeran Buhecha, Cambridge University
Advisor: **Bruce Stillman**
Sponsor: Burroughs Wellcome
Characterization of hORC1 ubiquitination.

Brianna Burden, University of California, Los Angeles
Advisor: **Linda Van Aelst**
Sponsor: Jephson Educational Trust
Molecular characterization of DOCK7.

John Calarco, University of Toronto
Advisor: **Adrian R. Krainer**
Sponsor: The Past URPs Fund
SMN alternative splicing and spinal muscular atrophy.

Carol Cho, Seoul National University
Advisor: **Leemor Joshua-Tor**
Sponsor: Emmanuel Ax Fund
The genetic switch: Elucidating the structural components of the GAL transcription system.

Boaz Gildor, Tel Aviv University
Advisor: **Eli Hatchwell**
Sponsor: Burroughs Wellcome
Gene expression analysis in putative centromere position-effect.

Katrina Gold, Cambridge University
Advisor: **Rob Martienssen**
Sponsor: Burroughs Wellcome
How are RNA-dependent RNA polymerases and Dicers involved in microRNA-based gene regulation?

Emily Heikamp, Duke University
Advisor: **Vivek Mittal**
Sponsor: Bliss Fund
A role for Id1 and Id3 in tumor angiogenesis.

Max Jan, Princeton University
Advisor: **David Jackson**
Sponsor: Steamboat Foundation
Characterization of a defect in protein trafficking in *Arabidopsis*.

Miranda Kim, Amherst College
Advisor: **W. Richard McCombie**
Sponsor: Glass Fund
Epigenetic modification in cancer.

Matthew Klein, Reed College
Advisor: **Roberto Malinow**
Sponsor: Libby Fund
How I failed to cure Alzheimer's disease in ten weeks.

Amy Leung, Cornell University
Advisor: **Yi Zhong**
Sponsor: Von Stade Fund
Dissecting the pathological effects of Aβ42 assemblies in the *Drosophila* Alzheimer's model.

Jakob Macke, Oxford University
Advisor: **Karel Svoboda**
Sponsor: Burroughs Wellcome
Tracking dynamics of synapses in the intact brain.

Pawel Mazur, Warsaw University
Advisor: **Marja Timmermans**
Sponsor: Burroughs Wellcome
Characterization of miRNA166 expression pattern during leaf dorsoventral patterning in *Arabidopsis*.

Carissa Meyer, Harvard University
Advisor: **Masaaki Hamaguchi**
Sponsor: Jephson Educational Trust
Cell-cycle-mediated growth suppression of breast cancer cells by *DBC2*.

Bao Pham, Trinity College
Advisor: **Ravi Sachidanandam**
Sponsor: Olney Fund
Staufen: A case study in evolution.

Siddharth Srivastava, Columbia University
Advisor: **Michael Q. Zhang**
Sponsor: Read Fund
Mapping pancreatic-specific promoters in zebrafish.

Eric Sullivan, Wesleyan University
Advisor: **Zachary Mainen**
Sponsor: Von Stade Fund
Is our children learning? Selective attention and set shifting in rodents.

Beatrice Tapawan, Mt. Holyoke College
Advisor: **Scott Lowe**
Sponsor: The Baltimore Family Fund
Suppression of target genes in the ATM-p53 pathway by RNAi.

Nicholas Wall, California Institute of Technology
Advisor: **Z. Josh Huang**
Sponsor: The Baltimore Family Fund
Development of dendritically targeted GABAergic synapses in the hippocampus and neocortex.

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 eight 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 2004–2005 Partners for the Future are:

Partner	High School	CSHL Mentor	Laboratory
Saroja Bangaru	Roslyn High School	Linda Wilbrecht	Karel Svoboda
Khadeejah Bari	Portledge School	Cordula Schulz	Yuri Lazebnik
Sarah Goldman	Roslyn High School	Masaaki Hamaguchi	
Christopher Hubley	Oceanside High School	Eli Hatchwell	
Noah Jakimo	Syosset High School	Ravi Sachidanandam	
Sean Kotkin	Herricks High School	Wolfgang Lukowitz	
Eric Paniagua	Massapequa High School	Mitya Chklovskii	
Adam Robbins	Oyster Bay High School	Dom Duelli	

(*First row, left to right*) Adam Robbins, Saroja Bangaru, Noah Jakimo, Khadeejah Bari, Eric Paniagua, Sarah Goldman. (*Second row, left to right*) Christopher Hubley, Sean Kotkin.

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and secondary school students the opportunity to acquire a greater knowledge and understanding of their environment. Through a series of specialized field courses, younger students can engage in introductory programs such as *Nature Bugs*, *Nature Detectives*, and *Nature Discovery*, and older students can enroll in more advanced programs such as *Marine Biology*.

During the summer of 2004, 379 students participated in 26 courses within the program. The classes were held outdoors, weather permitting, at the Southdown School. The Laboratory has equipped and maintains classroom and laboratory facilities at Southdown School. 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, and the Cold Spring Harbor Fish Hatchery and Aquarium, as well as in other local preserves and sanctuaries.

In addition to the three, 2-week sessions, the *Adventure Education* course met on Friday, July 16, for a 6-mile canoe trip on the Nissequogue River in Smithtown to navigate and explore the waters of Long Island. The course emphasizes plant and animal life indigenous to the area as well as historic points of interest.

This year, we offered *Nature Photography I* and *II*. The course 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. In *Photography II*, the students were introduced to digital photography. One of their projects was to make a photo collage of their pictures. We provided a computer, color printer, and a darkroom to further develop their skills.

PROGRAM DIRECTOR: William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR: Sharon Bense, Cold Spring Harbor Laboratory

INSTRUCTORS: **Jessica Badalucco,** B.S. in Biology, Adelphi University
Amy Friedank, B.S. in Marine Science, Long Island University, Southampton College
Jimmie Hamilton, Pratt Institute, Graphic Design/Photography
Ann Marie LaRuffa, B.A in Natural Science, Adelphi University
Michael Zarzicki, B.A. in English, Adelphi University

COURSES

Nature Bugs (Kindergarten): Exploration, games, stories, and dramatics are used to introduce the young child to the variety of natural habitats.

Nature Detectives (Grades 1–2): An introductory course in nature study, stressing interrelationships between plants and animals. A variety of habitats are explored.

Nature Discovery (Grades 1–2): Students continue their discovery of nature through activities and concepts.

Ecology Explorers (Grades 3–4): Natural communities, food webs, and the succession of communities are studied. Students study the diversity of plant and animal forms native to the Cold Spring Harbor area.

Pebble Pups (Grades 3–4): Elementary geology for students interested in making a basic study of rocks and minerals available on Long Island. Each student completes a rock and mineral collection. Dinosaurs and fossils are featured themes. Some of the highlights 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): Study of vertebrate, invertebrate, and plant life found in area bogs, ponds, lakes, and streams.

Nature Photography (Grades 5–7): Students take pictures and use the darkroom to learn techniques of printing and developing. Photographic techniques relating to nature photography are emphasized.

Adventure Education (Grade 6–10): A 6-mile canoe trip up and down the Nissequouge River explores the flora and fauna of the waterway.

Marine Biology (Grades 8–10): A more sophisticated study is offered of plants and animals native to the inner and outer harbors. This course provides field trips, dissection, use of the microscope, and laboratory experiments.

COLD SPRING HARBOR LABORATORY MEETINGS AND COURSES

ACADEMIC AFFAIRS

The academic program at the Laboratory serves to communicate new discoveries, concepts, and methodologies to an international community of scientists. The program consists of advanced laboratory and lecture courses, as well as large meetings and biotechnology conferences that are held almost year-round. Last year, almost 7000 scientists came from around the world to attend these events.

This year, 22 major meetings were held at the Laboratory, including the yearly Symposium. Although the meetings calendar is full with many meetings that recur on a 2-year cycle, there is always room for new conferences that represent rapidly moving areas of science. This year, successful new neurobiology conferences on *Computational and Systems Neuroscience* and *Channels, Receptors, and Synapses* were held. The latter meeting was organized by Robert Huganir, Lily Jan, and Morgan Sheng and is scheduled again for the 2006 season. Many of the meetings were oversubscribed, including the Symposium, *The Biology of Genomes*, *Retroviruses,* and *Translational Control* meetings. At most meetings, approximately 25% of the participants are graduate students, whereas an additional 25–30% are postdoctoral fellows. This indicates the extent to which the meetings are a popular venue for young scientists and it is at these meetings that many of them give their first presentations.

The 69th Symposium on “Epigenetics” featured 68 talks and 210 poster presentations. Opening night talks were delivered by Davor Solter, Barbara Meyer, David Allis, and Andrew Fire. Daniel Gottschling from the Fred Hutchinson Cancer Research Center closed the meeting with a summary. Gary Felsenfeld gave the Reginald G. Harris Lecture on “Chromatin Boundaries and Chromatin Domains.” The Dorcas Cumming Lecture, which is open to friends and neighbors of the Laboratory, was given by David Haig of Harvard University on the subject of “The Divided Self—Brains, Brawn, and the Superego.”

Twenty-eight lecture and laboratory courses were held, covering a diverse array of topics in neurobiology, structural biology, molecular biology, and genetics and bioinformatics. These courses allow the student to return home and immediately apply the latest relevant techniques to their own research. Courses are always being updated by the instructors to include new methodologies and directions. The success of the courses is due, in large part, to the skills, talents, and hard work of the faculty instructors, lecturers, and assistants who come from institutions around the world.

We must make note this year of several heroic instructors who have been teaching courses for 10 years or more. These include Dick Burgess and Sue-Hwa Lin, instructors for the spring course on *Protein Purification and Characterization*; Cary Lai and Jim Boulter for the *Advanced Techniques in Molecular Neuroscience* summer course; Bill Pearson and Randy Smith, instructors for the *Computational Genomics* fall course; Carlos Barbas and Gregg Silverman, who teach the course on *Phage Display of Protein and Peptides*; and the entire teaching faculty of the *X-ray Crystallography* course: Bill Furey, Gary Gilliland, Alex McPherson, and Jim Pflugrath.

The extensive offerings would not be possible without a series of grants from federal and private sources. These include the National Institutes of Health, the National Science Foundation, the Howard Hughes Medical Institute, the Esther and Joseph A. Klingenstein Fund, and the Eppley Foundation. Core support for the meetings program is provided by the Laboratory's Corporate Benefactors, Sponsors, Affiliates, and Contributors. In addition, the Laboratory receives valuable support from many companies that donate supplies and lend equipment for the courses.

The success of the very large number of meetings and courses is also due to the skilled work of many Cold Spring Harbor staff and faculty who contribute their expertise, efforts, and good humor to the program. Without their diligence, the programs would shine very much less brightly. We particularly acknowledge the efforts of Mike Cannavarro, our information technology specialist, and Jenna Williams, meeting administrator, who departed during the course of the year.

Terri Grodzicker
Assistant Director for Academic Affairs

David Stewart
Executive Director, Meetings & Courses

69TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Epigenetics

June 2–7 460 participants

ARRANGED BY **Bruce Stillman** and **David Stewart,** Cold Spring Harbor Laboratory

Until recently, the general view of heredity has been shaped by the lens of DNA. Indeed, the 2003 Symposium on "The Genome of *Homo Sapiens*" contributed to that view by emphasizing the importance of DNA sequence and its origins. But increasingly, investigators are exploring a set of secondary phenomena that give rise to heritable changes in gene function that occur without a change in the underlying DNA sequence—epigenetic mechanisms such as DNA methylation, histone acetylation, imprinting, RNA interference, gene silencing, and paramutation. A growing body of evidence indicates that epigenetic changes are important contributors to the pathogenesis of disease in humans, animals, and plants and may lie at the heart of many important gene-environment interactions. And so it seemed timely to hold a Symposium explicitly devoted to epigenetics.

Previous Symposia that have in part examined the role of the macromolecular context in which the primary genetic information is found include the 1941 Symposium on "Genes and Chromosomes: Structure and Organization," which emphasized a biophysical approach to these structures; the two closely separated Symposia that examined "Chromosome Structure and Function" (1973) and "Chromatin" (1977), at which latter meeting the nature of the nucleosome was unveiled; and the 1993 Symposium on "DNA and Chromosomes," by which time the Human Genome Project with its focus on the primary sequence was well under way. The 69th Symposium, however, was the first to fully explore the heritable aspects to these and related biochemical phenomena.

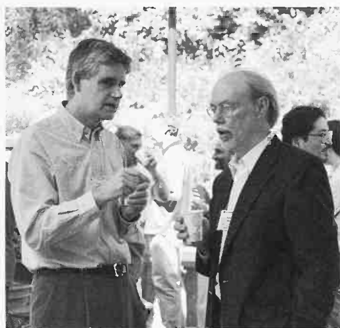
The field of epigenetics as we know it today was prominently introduced at the 1951 Symposium on "Genes and Mutations." There, Ed Lewis presented data on position effect variegation in *Drosophila*, a phenomenon that has played an important role in the history of the field. Equally importantly, Barbara McClintock presented her ideas about heterochromatin and movable genetic elements, the so-called Ac-Ds system in maize that opened up understanding of transposition and its links to gene silencing and heterochromatin. This year's Symposium witnessed the first molecular description of her ideas, including links to RNAi.

D. Haig, Dorcas Cummings Lecture

The 69th Symposium ran for five days and included 460 participants with 68 oral presentations and 210 poster presentations. We wish to thank Dr. David Haig for his superb Dorcas Cummings Memorial public lecture presenting a theoretical approach to epigenetics on the subject of *The Divided Self—Brains, Brawn, and the Superego.* We also wish to thank the first night speakers, Drs. Davor Solter, Barbara Meyer, David Allis, and Andrew Fire for their superb overview presentations, and we particularly thank Dr. Daniel Gottschling for agreeing to summarize the Meeting.

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:* Amgen Inc.; Aventis Pharma AG; Bristol-Myers Squibb Company; Eli Lilly and Company; GlaxoSmithKline; Novartis Institutes for

BioMedical Research; Pfizer Inc. *Corporate Sponsors:* Applied Biosystems; AstraZeneca; BioVentures, Inc.; Cogene BioTech Ventures, Ltd.; Diagnostic Products Corporation; Forest Laboratories, Inc.; Genentech, Inc.; Hoffmann-La Roche Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Kyowa Hakko Kogyo Co., Ltd.; Lexicon Genetics, Inc.; Merck Research Laboratories; New England Biolabs, Inc.; OSI Pharmaceuticals, Inc.; Pall Corporation; Schering-Plough Research Institute; Wyeth Genetics Institute. *Plant Corporate Associates:* ArborGen; Monsanto Company. *Corporate Affiliates:* Affymetrix, Inc.; Agencourt Biosciences Corporation. *Corporate Contributors:* Axxora; Biogen, Inc.; EMD Bioscience; Epicentre; Technologies, Illumina; Integrated DNA Technologies; IRx Therapeutics; KeyGene. *Foundations:* Albert B. Sabin Vaccine Institute, Inc.



B. Stillman, P. Sharp

Catching the live broadcast in Grace Lobby

D. Solter, W. Herr

S. Emmons, G. Ruvkun, B. Meyer

PROGRAM

Introduction
B. Stillman, *Cold Spring Harbor Laboratory*

Nuclear Reprogramming, Chromosome Inactivation, and Imprinting
Chairperson: A. Bird, *University of Edinburgh, United Kingdom*

Small RNAs I
Chairperson: P. Sharp, *Massachusetts Institute of Technology, Cambridge*

DNA Methylation: Mechanism and Regulation
Chairperson: A. Surani, *Wellcome Trust/Cancer Research United Kingdom, Cambridge*

Reginald G. Harris Lecture: Position Effects and Nuclear Organization
Chairperson: F. Felsenfeld, *National Institutes of Health/NIDDK, Bethesda, Maryland*

Position Effects and Nuclear Organization
Chairperson: G. Almouzni, *Institut Curie Recherche, Paris, France*

Chromatin Inheritance and Assembly
Chairperson: M. Kuroda, *Harvard Medical School, Boston, Massachusetts*

Epigenetic Regulation of Gene Expression
Chairperson: V. Chandler, *University of Arizona, Tucson*

Histone Modifications
Chairperson: J. Lee, *Massachusetts General Hospital, Boston*

Epigenetic Twists
Chairperson: D. Stewart, *Cold Spring Harbor Laboratory*

Dorcas Cummings Lecture: The Divided Self: Brains, Brawn, and the Superego
Speaker: David Haig, *Harvard University, Cambridge, Massachusetts*

Small RNAs II
Chairperson: S. Henikoff, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

Epigenetics and Disease
Chairperson: M. Grunstein, *University of California, Los Angeles*

Summary
Daniel Gottschling, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

J. Watson, R. Wickner, J. Witkowski

MEETINGS

Systems Biology: Genomic Approaches to Transcriptional Regulation

March 4–7 242 participants

ARRANGED BY **Stephen Small,** New York University
Gary Stormo, Washington University
Michael Zhang, Cold Spring Harbor Laboratory

Deciphering the network of regulatory interactions that control development and responses to the environment is one of the major challenges in modern molecular biology. Complete genome sequences and a variety of new technological advances provide copious data relevant to the discovery of the network components and connections. Optimum utilization of these data requires a concerted effort by experimental and computational biologists, each bringing their own expertise to the problem, but interacting vigorously to ensure that the right problems are being addressed and the best methods are being employed. This second meeting brought together both groups, covering a range of model organisms, biological questions, and experimental and computational approaches, for a highly cross-disciplinary and interactive forum. Sessions described state-of-the-art methods for analyzing protein-DNA interactions and identifying the network connections on whole-genome scales. The newest high-throughput techniques, and some of the most recent findings from those studies, were described. Two sessions emphasized the latest computational methods for analyzing the data, including approaches that simultaneously take advantage of the experimental results from one organism and the conserved genomic sequences from additional organisms to help identify the most fundamental, conserved features in the genomes. A final session on emerging techniques closed the meeting. Numerous posters were presented, and lively, informal discussions were ongoing throughout the meeting. It was clear that significant progress in both experiments and computational analyses has taken place even since the previous meeting a year ago. A planning session was held, and a steering committee was appointed to foster similar interactions in the future.

G. Stormo, N. Gershenzon, A. Sengupta

PROGRAM

Keynote Address: A Draft Transcriptional Regulatory Code for Yeast
R. Young, *Whitehead Institute/Massachusetts Institute of Technology, Cambridge*

Transcriptional Regulation and Genomics
Chairpersons: S. Small, *New York University;* R. Young, *Whitehead Institute/Massachusetts Institute of Technology, Cambridge*

Computational Approaches to Identifying Regulatory Elements in Genomic DNA
Chairpersons: P. Benfey, *Duke University, Durham, North Carolina;* C. Lawrence, *Wadsworth Center, Albany, New York*

Transcription Networks
Chairpersons: J. Reinitz, *Stony Brook University, New York;* A. Keating, *Massachusetts Institute of Technology, Cambridge*

Experimental Approaches to Genome-wide Transcriptional Regulation
Chairpersons: M. Johnston, *Washington University School of Medicine, St. Louis, Missouri;* J. Kadonaga, *University of California, San Diego*

Computational II and Comparative Genomics
Chairpersons: M. Zhang, *Cold Spring Harbor Laboratory;* M. Tompa, *University of Washington, Seattle*

Emerging Technologies
Chairpersons: E. Davidson, *California Institute of Technology, Pasadena;* G. Stormo, *Washington University School of Medicine, St. Louis, Missouri*

Keynote Address: Functional Properties of the Gene Regulatory Network for Early Sea Urchin Development
E. Davidson, *California Institute of Technology*

C. Tang, M. Kloster

S. Lincoln, B. Aronow, D. Slonim

R. Lake, A. Nagy

Computational and Systems Neuroscience (COSYNE)

March 24–28 329 participants

ARRANGED BY **Carlos Brody,** Cold Spring Harbor Laboratory
Alexander Pouget, University of Rochester
Michael Shadlen, University of Washington
Tony Zador, Cold Spring Harbor Laboratory

Systems neuroscience focuses on how systems of many connected neurons operate and demands both experimental and theoretical/computational approaches in its efforts to understand brain function. The latter are used to both analyze the rich, complex data the experiments produce and to help formalize—and thus more rigorously explore—proposed hypotheses regarding mechanisms underlying the phenomenology. A meeting combining equal contributions from both experimental and computational investigators was heretofore lacking. The first *Computational and Systems Neuroscience* meeting provided such a forum. Judging from the highly enthusiastic response of the participants, COSYNE was successful in providing a fruitful venue for abundant cross-disciplinary interaction. Topics ranged from sensory processing to the neuroscience of cognition, and all topics blended together presentations that put forth experimental data, theoretical principles, and sophisticated data analysis methods.

Major funding for this meeting was provided by the Swartz Foundation and the Redwood Neuroscience Institute. Funding was also provided in part by the National Science Foundation and the National Institute of Mental Health, a branch of the National Institutes of Health. Additional support was provided by Advanced Acoustic Concepts, Inc.

Pre-COSYNE Workshop 1: Auditory Processing of Vocalizations and Other Complex Sounds

March 24 40 participants

ARRANGED BY **Tony Zador,** Cold Spring Harbor Laboratory

There has been increasing interest in the computational and experimental analysis of the mechanisms by which complex sounds such as animal vocalizations are processed in the nervous system. This one-day workshop on processing of complex sounds addressed a number of important questions, including (1) How far can we go with linear models? What is the best basis for describing complex sounds? How can it be found? (2) What processing is done at each level of the auditory pathway? (3) What role do sparse representations have in auditory processing? (4) Does the nervous system treat conspecific vocalizations differently from other complex sounds? (5) How distinct are avian and mammalian strategies for processing complex sounds? (6) What role does behavioral context have?

A. Zador

M. Long, C. Weaver

Pre-COSYNE Workshop 2: Neurobiology of Decision Making

March 24 56 participants

ARRANGED BY **Carlos Brody,** Cold Spring Harbor Laboratory
Michael Shadlen, University of Washington
Xiao-Jing Wang, Brandeis University

How are decisions made in the brain? Both psychologists and neuroscientists have addressed this question, often by using simple two-alternative decision tasks in the lab (e.g., asking subjects to decide "is this light bright or dark?"). In this workshop, we explored the surprising, and very exciting, convergence between the mathematical frameworks built by psychologists to describe the behavior of whole subjects, and the frameworks built by neuroscientists to describe the behavior of neurons recorded from animals performing two-alternative decision tasks. The convergence of methods and results, starting from two very different approaches, suggests that we may be close to an understanding of the key principles and neural mechanisms behind simple decision-making behaviors.

Pre-COSYNE Workshop 3: Microcircuitry of Cortical Columns

March 23–24 65 participants

ARRANGED BY **Dmitry Chklovskii,** Cold Spring Harbor Laboratory
Armen Stephanyants, Cold Spring Harbor Laboratory

The microcircuitry of the *Cortical Column* workshop gathered researchers who use a variety of anatomical, physiological, genetic, and computational approaches to study cortical connectivity. Despite different techniques, there was a strong convergence of ideas on a number of themes. In particular, understanding of the roles of specificity and randomness in synaptic connectivity received much attention.

V. Uzzell, K. Briggman, J. Shlensi

J. Kretzberg, A. Schaefer

Evolution of Developmental Diversity

March 31–April 4 96 participants

ARRANGED BY **Richard Behringer,** University of Texas/M.D. Anderson Cancer Center
Nipam Patel, HHMI/University of California, Berkeley

This second meeting attracted approximately 100 researchers from around the world. It brought together a diverse group of scientists studying various molecular genetic and developmental aspects of animal and plant biology with a strong emphasis on morphological and functional comparisons of organismal diversity and evolution.

The meeting opened with a keynote lecture given by Fred Nijhout (Duke University) on the evolution of developmental mechanisms. There were eight sessions whose topics reflected the most advanced and interesting areas of study in the evolution and development field, including genetics, the evolution of gene regulation, genomics, axial pattern, segmentation and regionalization, speciation/microevolution and sexual differentiation, and organogenesis/morphological innovation. Each session had two invited talks from senior researchers. Between these more in-depth talks, six speakers per session were selected from the abstracts for more concise presentations. The meeting also included the presentation of posters in one afternoon session.

Research utilizing the primary animal and plant model systems to understand developmental mechanisms has made great strides over the last few decades, demonstrating a strong molecular and cellular understanding of conserved developmental pathways. These model systems have provided a now classical framework for studies in other plants and animals. However, it is clear that the very few primary model organisms are not sufficient to understand the tremendous diversity of animal and plant life on earth. Understanding the generation of this diversity, honed over eons of evolution, most likely will provide important insights into the mechanisms that cause organ variation and the evolution of species. These insights should provide novel information for understanding human health and disease mechanisms. Thus, it is now clear that other animal and plant models must be used to complement studies utilizing the primary model organisms. Studies of these new model organisms have been greatly facilitated by genome and cDNA sequencing projects, transgenesis, and new gene knockdown technologies. The use of diverse organisms to study biological questions was the universal theme of the meeting with talks on alligator, Amphioxus, annelid, ascidian, bat, butterfly, centipede, cichid fish, *Coqui*, cricket, crustacean, hemichordate, jelly fish, leech, medaka, monkey flower, nematode, opossum, *Planaria*, sea urchin, spider, starfish, tribolium, wallaby, wasp, and others.

R. Behringer, E. Pennisi

PROGRAM

Keynote Address: The Evolution of Developmental Mechanisms
F. Nijhout, *Duke University*

Genomics I
Chairperson: A. Sánchez, *University of Utah School of Medicine, Salt Lake City*

Segmentation/Regionalization
Chairperson: S. Brown, *Kansas State University, Manhattan*

Axial Pattern
Chairperson: J. Gerhart, *Harvard Medical School, Boston, Massachusetts*

Speciation / Microevolution and Sexual Differentiation
Chairperson: A. Sinclair, *University of Melbourne, Royal Children's Hospital, Australia*

Genetics I
Chairperson: S. Hake, *University of California, Berkeley*

Organogenesis / Morphological Innovation
Chairperson: L. Holland, *University of California, San Diego*

Genetics II
Chairperson: R. Sommer, *Max-Planck Institute for Developmental Biology, Tübingen, Germany*

Evolution of Gene Regulation
Chairperson: C. Amemiya, *Benaroya Research Institute at Virginia Mason, Seattle, Washington*

S. Alvarado, W. Jeffrey

N. Patel, A. Burke

R. Palmer, P. Brakefield, F. Nijhout

Channels, Receptors, and Synapses

April 21–25 238 participants

ARRANGED BY **Richard Huganir,** HHMI/Johns Hopkins University School of Medicine
Lily Jan, HHMI/University of California, San Francisco
Morgan Sheng, HHMI/Massachusetts Institute of Technology

The brain consists of a vast network of excitable cells (neurons) that conduct electrical impulses and communicate with each other via specialized junctions (synapses). Information is processed and stored in the nervous system through patterns of electrical activity and via changes in the strength and structure of synapses. As master regulators of neuronal excitability and synaptic communication, ion channels and receptors lie at the heart of neurobiology. In recent years, molecular and cell biological analyses of neuronal ion channels and receptors have revolutionized our understanding of the basic mechanisms that control electrical signaling and synaptic function in the nervous systems. The convergence of advances in biochemistry, molecular genetics, microscopic imaging, and electrophysiology has made this interdisciplinary field one of the most exciting and rapidly growing in neuroscience. Several landmark advances have transformed the phenomenology in this field into underlying molecular mechanisms, including the genomic cataloging of all channels and receptors, the discovery of the three-dimensional "crystal" structure of a membrane ion channel, the identification of specific anchoring proteins that bind to the cytoplasmic tails of ion channels and receptors, and the discovery of dynamic and regulated trafficking of receptors/ion channels. The fundamental aspects of these mechanisms seem to be evolutionarily conserved from primitive nervous systems of worms to the human brain.

M. Wampler, S. Thompson

The entire field is poised for further breakthroughs that will not only illuminate basic workings of the brain, but also shed light on neurological and psychiatric diseases that stem from abnormal neuronal excitability and synaptic dysfunction. Such breakthroughs will be facilitated by cross-fertilization of ideas and technologies between scientists studying related questions in diverse organisms using different methodological approaches. To provide a forum that unites this exciting multidisciplinary area, a new CSHL meeting on *Channels, Receptors, and Synapses* was started in April 2004.

The inaugural meeting was extremely successful, bringing together nearly 240 participants from United States, Europe, and Asia in an atmosphere of social and scientific exchange. A wide range of topics were discussed, including the structure and function of ion channels and receptors, new classes of channels/receptors, regulation of ion channel/receptor function by phosphorylation, membrane trafficking and degradation; molecular mechanisms of synaptic transmission/plasticity and their roles in brain function; and the involvement of ion channels/receptors in human disease. More than 60 attendants were selected to give

M. Kennedy, W. Ford, H.H. Beale, H. Carlisle, A. Tapper

oral presentations of their work, while the majority of the rest presented posters. Special lectures were given by Rod MacKinnon, 2003 Nobel laureate, on the three-dimensional structures of ion channels, and by Roberto Malinow (CSHL professor), on trafficking of glutamate receptors in synaptic plasticity. The overall response to the meeting was extremely positive, especially given that this was the first conference. Participants particularly enjoyed the breadth of subjects covered and the chance to interact with investigators in related but distinct fields. The opportunity for junior investigators (including postdocs and students) to present their own work was especially applauded. Based on the success of the first meeting, this conference will take place again in two years. It is anticipated that this Cold Spring Harbor meeting will become a "must-attend" conference for learning the latest developments in molecular and cellular physiology of the brain.

PROGRAM

Modulation of Ion Channels and Receptors
Chairpersons: S. Moss, *University of Pennsylvania, Philadelphia*; A. Dolphin, *University College London, United Kingdom*

Ion Channels and Receptors in Brain Function and Disease
Chairpersons: M. Mishina, *Tokyo University, Japan*; L. Ptacek, *University of Utah, Salt Lake City*

Special Lecture: Three-dimensional Structure of Ion Channels
R. MacKinnon, *The Rockefeller University, New York*

Structure and Function of Ion Channel/Receptors
Chairpersons: D. Clapham, *Children's Hospital, Boston, Massachusetts;* G. Yellen, *Harvard Medical School, Boston, Massachusetts*

Presynaptic Mechanisms
Chairpersons: T. Sudhof, *University of Texas Southwestern Medical Center, Dallas;* R.W. Tsien, *Stanford University, California*

Postsynaptic Signaling Mechanisms
Chairpersons: M. Greenberg, *Children's Hospital, Boston, Massachusetts;* M. Kennedy, *California Institute of Technology, Pasadena*

Synaptic Plasticity
Chairpersons: R. Malenka, *Stanford University, Palo Alto, California;* G. Turrigiano, *Brandeis University, Waltham, Massachusetts*

Special Lecture: Receptor Trafficking in Synaptic Plasticity
R. Malinow, *Cold Spring Harbor Laboratory*

Development of Synapses
Chairpersons: H. Cline, *Cold Spring Harbor Laboratory;* M. Bear, *Massachusetts Institute of Technology, Cambridge*

Targeting, Trafficking, and Degradation of Ion Channels/Receptors
Chairpersons: G. Westbrook, *Vollum Institute, Portland, Oregon;* J. Kaplan, *Massachusetts General Hospital, Boston*

M. Grunnet, O. Uchitel

H. Kataoka, L. Jan

D. Clapham, A. Maffei

Gene Expression and Signaling in the Immune System

April 28–May 2 294 participants

ARRANGED BY **Doreen Cantrell,** University of Dundee
Richard Flavell, HHMI/Yale University
Rudolf Grosschedl, University of Munich
Stephen Smale, HHMI/University of California School of Medicine

This second meeting in the series attracted more than 290 participants from around the world. It maintained its unique emphasis on mechanistic studies that most significantly advance our understanding of the gene regulation and signal transduction circuitry within the immune system, as well as the use of the immune system as a tool for elucidating fundamental molecular processes. Invited and oral presentations selected from the abstracts covered a broad range of topics concerning developmental regulation of the immune system and immune responses to infection. One highlight of the meeting was a series of presentations that are beginning to firmly define the molecular events that regulate cell fate decisions as hematopoietic stem cells choose between various lymphocyte and myeloid cell lineages. Another highlight was a spirited discussion of the activation-induced deaminase (AID) protein that is essential for both somatic hypermutation and class switching of immunoglobulin genes. Conflicting evidence concerning potential roles of AID in RNA processing or, alternatively, direct deamination of nucleotides within variable regions and switch regions during hypermutation and switching was debated. Other central topics included the epigenetic regulation of development, mechanisms involved in the T helper 1–T helper 2 lineage decision, mechanisms of tolerance induction, and Toll-like receptor signaling mechanisms.

Special thanks to the following organizations for support of this meeting: Abbott Laboratories, Biogen IDEC, Boehringer Ingelheim Pharmaceuticals, Inc., Hoffmann-La Roche, Merck & Co., Inc., and Pfizer. This meeting was funded in part by the National Institute of Allergy and Infectious Diseases, a branch of the National Institutes of Health.

S. Smale, R. Flavell, R. Grosschedl

R. Sen, A. Roy, Z. Desgranges

Stem Cells and Early Development Decisions
Chairperson: I. Weissman, *Stanford University School of Medicine, California*

Regulation of Immune Cell Development
Chairperson: K. Calame, *Columbia University College of Physicians & Surgeons, New York*

Antigen Receptor Gene Assembly, Somatic Hypermutation, and Class Switching
Chairperson: F. Alt, *HHMI/Children's Hospital, Boston, Massachusetts*

Chromatin Structure and Epigenetic Regulation
Chairperson: A. Tarakhovsky, *The Rockefeller University, New York*

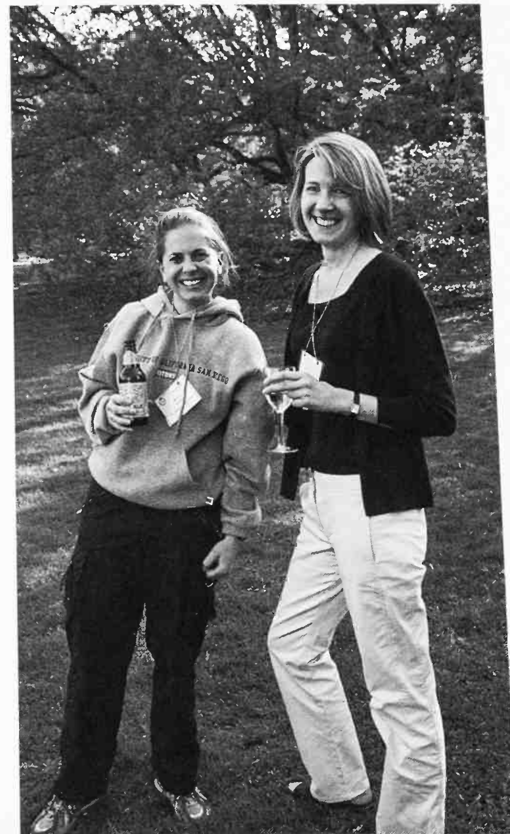
Lymphocyte Differentiation
Chairperson: L. Glimcher, *Harvard School of Public Health, Boston, Massachusetts*

Signal Transduction in Immune Cells
Chairperson: D. Cantrell, *University of Dundee, United Kingdom*

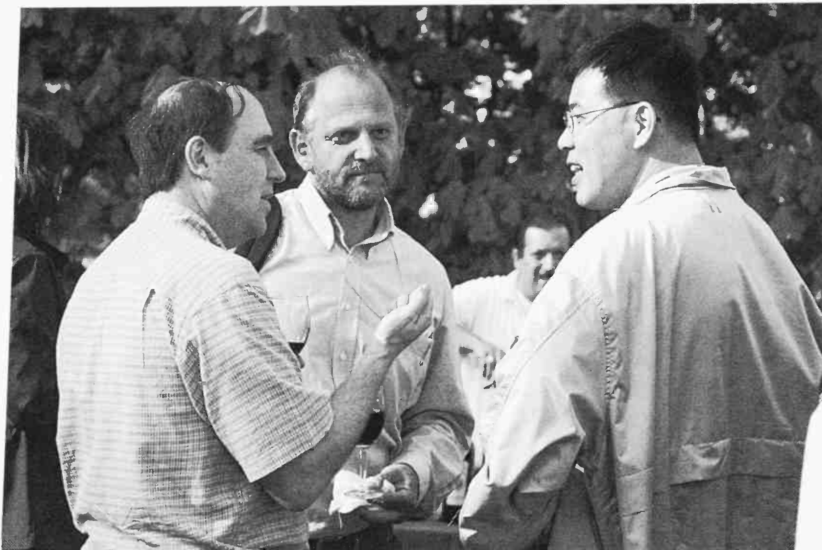
Regulation of Lymphocyte Function I
Chairperson: C. Benoist, *Joslin Diabetes Center, Boston, Massachusetts*

Regulation of Lymphocyte Function II
Chairperson: S. Ghosh, *Yale University School of Medicine, New Haven, Connecticut*

J. Madrenas, C. Bueno



K. Mowen, S. Szabo



X. Lin, G. Koretzky, M. Reth

Molecular Chaperones and Heat Shock Response

May 5–9 298 participants

ARRANGED BY **James Bardwell,** University of Michigan
Elizabeth Craig, University of Wisconsin
Jonathan Weissman, University of California, San Francisco

This meeting, 22 years after the inaugural meeting, featured advances in the areas of structure and mechanism of action of molecular chaperones; mechanisms of induction of the stress response; the role of chaperones in protein degradation; and chaperone function in disease and aging. In the first area, further advances were described for the classic heat shock proteins, the GroEL/ES, the small heat shock factors, Hsp70, 90, and 100 classes, as well as more recently identified chaperones such as CsA in eubacteria. In addition, the first high-resolution structure of the Ero1 protein, which has an essential role in oxidative protein folding in eukaryotes, was presented. In a session of particular interest, the relationship between chaperone function and aging was featured with talks presenting experiments in *Caenorhabditis elegans* and *Drosophila* establishing a role of chaperone induction in extending lifespan, as well as the effects of aging on the propensity to form intracellular toxic protein aggregates. Several presentations revealed advances in our understanding of the function of ATP-dependent regulatory subunits such as the proteosome regulatory particle in eukaryotes and the related ClpA complex in bacteria in catalyzing the unfolding of proteins prior to their degradation. In the area of regulation of stress responses, a focus of the meeting was the advances in our understanding of the mechanism and biological consequences of the quality control machinery that prevents the secretion and ultimately degrades misfolded proteins in the endoplasmic reticulum (ER) as well as the unfolded protein response which induces a multifaceted response when misfolded proteins accumulate in the ER. In addition, progress toward an understanding of the function of heat shock transcription factors in complex organisms was discussed. In the area of protein misfolding and disease, a number of talks dealt with progress in the use of model systems in yeast, mice, and worms to attack this complex problem. The role

B. Bukau, A. Horwich, E. Craig

S. Gupta, J. Bardwell, D. Cyr, W. Antoine

N. Benaroudj, O. Kandror

oligomeric intermediates, which are often formed transiently during protein aggregation, in contributing to cellular toxicity and disease was also discussed. Finally, a number of talks focused on the biogenesis and maintenance of prions in yeast, and the roles that different chaperones have in this process including evidence that Hsp104 promotes prion propagation by acting to divide seeds, thereby allowing the creation of more infectious prion units. Thus, rather paradoxically, a chaperone can have the net effect of promoting protein misfolding.

This meeting was funded in part by the National Institute of Neurological Disorders & Stroke, and the National Institute of Child Health and Human Development, branches of the National Institutes of Health; Merck & Co., Inc., and the Alzheimer's Association.

PROGRAM

Diseases of Protein Misfolding
Chairperson: S. Lindquist, *Whitehead Institute, Cambridge, Massachusetts*

Quality Control and Protein Trafficking
Chairperson: R. Kopito, *Stanford University, California*

Cellular Response to Stress
Chairperson: C. Gross, *University of California, San Francisco*

Chaperone Function in Disease, Aging, and Development
Chairperson: R. Morimoto, *Northwestern University, Evanston, Illinois*

Regulation of the Stress Response
Chairperson: D. Ron, *New York University School of Medicine*

Chaperones and Proteolysis
Chairperson: T. Baker, *HHMI/Massachusetts Institute of Technology, Cambridge*

Chaperone Biochemistry and Protein Folding
Chairperson: B. Bukau, *Universität Heidelberg, Germany*

P. Bross, S. Vang

P. Carrigan, D. Smith, B. Matts

The Biology of Genomes

May 12–16 504 participants

ARRANGED BY **Thomas Hudson,** McGill University
Svante Pääbo, Max-Planck Institute
Jane Rogers, The Sanger Center
Edward Rubin, Lawrence Berkeley National Laboratory

This annual meeting was the 15th 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 the publication of a finished and draft sequences of the human, mouse, and rat genomes, in addition to several model organisms and hundreds of bacteria. Just over 500 people from around the world attended the meeting, with 331 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. 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. An ELSI (Ethical, Legal, and Social Implications) panel discussion focused on issues surrounding the process of community engagement in large-scale population genomic studies, using the case study of the Yoruba population of Ibadan Nigeria and the HapMap project.

The major themes of the meeting included the analysis of large-scale variation in genetic variation across the human genome (a product of the International HapMap Consortium), annotation of the human genome using multispecies sequence comparisons, and a myriad of large-scale approaches to understand complex biological processes. The Friday afternoon keynote talks were delivered by Stuart Schreiber and Craig Venter.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health.

S. Pääbo, E. Rubin, T. Hudson

PROGRAM

Comparative Genomics
Chairpersons: K. Peichel, *Fred Hutchinson Cancer Research Center, Seattle, Washington;* A. Sidow, *Stanford University, California*

Variation Technology and Haplotype Structures
Chairpersons: D. Altshuler, *Massachusetts General Hospital, Harvard Medical School, Boston;* M. Przeworski, *Brown University, Providence, Rhode Island*

High-throughput Biology
Chairpersons: A. Bradley, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* M. Snyder, *Yale University, New Haven, Connecticut*

Nonhuman Species and Environmental Genomics
Chairpersons: D. Relman, *Stanford University, California;* D. Rokhsar, *DOE Joint Genome Institute, Walnut Creek, California*

ELSI Panel Discussion
Moderator: J. McEwen, *National Human Genome Research Institute, Bethesda, Maryland*
Panelists: C. Adebamowo, *University of Ibadan, Nigeria, Africa;* P. Marshall, *Case Western Reserve University, School of Medicine, Cleveland, Ohio;* C. Rotimi, *Howard University, Washington, D.C.;* C. Royal, *Howard University, Washington, D.C.*

Computational Genomics
Chairpersons: P. Bork, *European Molecular Biology Laboratory, Heidelberg, Germany;* L. Pachter, *University of California, Berkeley*

Polymorphisms and Their Relationship to Biology
Chairpersons: J. Kere, *Karolinska Institutet, Huddinge, Sweden;* K. Frazer, *Perlegen Sciences, Mountain View, California*

Keynote Speakers
S. Schreiber, *Harvard University, Cambridge, Massachusetts;* C. Venter, *The Center for the Advancement of Genomics, Rockville, Maryland*

Genome Structure and Evolution
Chairpersons: W.-H. Li, *University of Chicago, Illinois;* C.-I. Wu, *University of Chicago, Illinois*

T. Andersson, J. Larkin

J. Hancock, P. Little, S. Brown

J. Kent, L. Stein

K. Kimura, Y. Suzuki, A. Fujiyama

The Cell Cycle

May 19–23 341 participants

ARRANGED BY **Orna Cohen-Fix,** National Institutes of Health/NIDDK
Stephen Elledge, HHMI/Harvard University
J. Wade Harper, Harvard Medical School
David Morgan, University of California, San Francisco

This 8th biannual meeting attracted participants from around the world and is internationally recognized for its ability to bring together scientists who study cell cycle regulation in eukaryotes ranging from yeast to humans. We were particularly fortunate this year to have Kim Nasmyth as our keynote speaker. Dr. Nasmyth, an acknowledged leader in the yeast cell cycle regulation field, opened the meeting with a wide-ranging and exciting talk describing the discovery of the separin-cohesin pathway and its role in the control of the metaphase to anaphase transition in eukaryotes ranging from yeast to mammals. Three broad themes were dominant throughout the meeting: the interaction of cell cycle control with developmental and cancer biology; the mechanisms of action of cell cycle checkpoints and their integration with signal pathways that control mitotic transitions; and the emerging use of high-throughput and whole-genome approaches to uncover new aspects of cell biology. Research in proteolysis for both G_1/S and G_2/M regulation in particular continues to receive intense focus, with much progress reported. As in previous years, there was also emphasis on the long-standing problem of regulation of DNA replication in the cell cycle, its onset, and its restriction to once per cell cycle. Significant insight into the conservation of transcriptional regulatory pathways involved in the G_1/S transition was presented when it was found that the G. cyclins phosphorylate and inactivate a repressor of the S-phase transcription factors. This is analogous to the Rb/E2F switch in mammals. New to the discussion was a more detailed understanding of how different Cdk/cyclin complexes choose their substrates during the cell cycle, with more than one surprise in that area. There was also an emphasis on the use of emerging technologies, both in tissue culture cells and in live animals such as *Caenorhabditis elegans*, to study the dynamics of signaling pathways and rapid changes in the localization of components of the mitotic apparatus in response to checkpoint activation. As in other years, scientists studying cell cycle regulation in yeast, *Xenopus*, *Drosophila*, nematodes, and mammals were well-represented. Once again, the striking phylogenetic conservation of cell cycle regulatory mechanisms was readily evident. This meeting also served to demonstrate the impact of whole-genome analysis on basic cell biology, and these achievements will no doubt continue to drive rapid

O. Cohen-Fix, S. Elledge, W. Harper, D. Morgan

progress in this field. The cell cycle conference was another landmark meeting for the cell cycle field, and the participants all continued to look forward to equally exciting meetings in future years.

This meeting was funded in part by the National Cancer Institute and the National Institute on Aging, branches of the National Institutes of Health.

PROGRAM

Cell Cycle Control Mechanisms
Chairperson: F. Cross, *The Rockefeller University, New York*

G_1 Control I
Chairpersons: N. Dyson, *Massachusetts General Hospital Cancer Center, Charlestown;* M. Tyers, *Samuel Lunenfeld Research Institute, Toronto, Canada*

G_1 Control II
Chairpersons: J. Roberts, *Fred Hutchinson Cancer Research Center, Seattle, Washington;* G. Hannon, *Cold Spring Harbor Laboratory*

S Phase
Chairpersons: J. Li, *University of California, San Francisco;* J. Diffley, *Imperial Cancer Research Fund, South Mimms, United Kingdom*

M Phase I
Chairpersons: J. Pines, *University of Cambridge, United Kingdom;* C. Rieder, *Wadsworth Center, Albany, New York*

M Phase II
Chairpersons: A. Amon, *Massachusetts Institute of Technology, Cambridge;* K. Gould, *HHMI/Vanderbilt University, Nashville, Tennessee*

Checkpoints I
Chairpersons: A. Carr, *University of Sussex, United Kingdom;* D. Toczyski, *University of California, San Francisco*

Checkpoints II
Chairpersons: S. Biggins, *Fred Hutchinson Cancer Research Center, Seattle, Washington;* H. Yu, *University of Texas Southwestern Medical Center, Dallas*

M. Saito, A. Taylor, M. Polymenis, R. Pathak

A. Devault, J. Li

M. Tyers, V. Duronio

Retroviruses

May 25–30 442 participants

ARRANGED BY **Eric Freed,** NCI-Frederick
Susan Ross, University of Pennsylvania

This 29th annual CSHL meeting originated in 1975 as a meeting on RNA Tumor Viruses and evolved to its current focus on Retroviruses in 1993. In our view, the 29th meeting was highly successful. The short oral presentations and posters were complemented by a keynote talk from Dr. Neil Copeland, who presented important findings from his and Nancy Jenkins' lab regarding the use of high-throughput analysis to map retroviral integration sites in tumors induced by murine leukemia viruses. The Jenkins and Copeland labs have used infection of various hematopoietic stem cells followed by transplantation into lethally irradiated mice to identify not only novel proto-oncogenes, but also cooperating cancer genes, tumor suppressors, and genes involved in stem cell transformation and immortalization. Although the topic of the keynote address was somewhat outside the mainstream of the conference, the talk was well-received.

There were 332 abstracts this year, with oral presentations arranged into 11 sessions, and three poster sessions. The oral sessions began with the entry phase of the replication cycle, proceeded with postentry events, assembly and release, integration, reverse transcription, pathogenesis/host factors, RNA-related events (transcription, processing, export, and packaging), and finished with antivirals. Although the most striking developments this year involved postentry events and assembly/release, 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 the lentiviral Vif protein counteracts the antiviral effects of the cellular enzyme APOBEC3G and novel findings relating to the identification of TRIM5alpha as the factor restricting postentry events in HIV-1 infection of nonhuman primate cells.

In other matters, the pattern of arranging posters alphabetically was continued this year. The cuisine continues at the enjoyable level noted for last several years, a big change from previous years. Posters were held in the evening this year after the wine and cheese party, rather than talks, which are difficult for some to manage after wine and beer. The Sunday morning session itself continues to be a bit of a problem as it is difficult for people to stay on, get up, and/or be enthusiastic at this final session. Finally, we very much appreciated the help and guidance of the CSHL staff, who made the meeting easy and enjoyable to organize. One final notable development: This year, we were asked to write a review of the meeting for the new on-line BioMed Central journal *Retrovirology*. The review is available at http://www.retrovirology.com/content/1/1/25.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided core support for this meeting.

S. Ross, E. Freed

P. Jolicoeur, D. Derse

PROGRAM

Entry I
Chairpersons: D. Lindemann, *Technische Universität Dresden, Germany;* L. Albritton, *University of Tennessee Health Sciences Center, Memphis*

Entry II
Chairperson: H. Garoff, *Karolinska, Institutet, Huddinge, Sweden*

Postentry I
Chairperson: P. Bieniasz, *Aaron Diamond AIDS Research Center, New York*

Postentry II
Chairpersons: C. Aiken, *Vanderbilt University, Nashville, Tennessee;* G. Towers, *University College London, United Kingdom*

Assembly/Release I
Chairpersons: X.F. Yu, *Johns Hopkins University School of Public Health, Baltimore, Maryland;* R. Montelaro, *University of Pittsburgh School of Medicine, Pennsylvania*

Keynote Speaker
Neal Copeland, *NCI-Frederick*

Integration
Chairpersons: H. Levin, *National Institutes of Health, Bethesda, Maryland;* D. Grandgenett, *St. Louis University Health Sciences Center, Missouri*

Assembly/Release II
Chairpersons: P. Spearman, *Vanderbilt University School of Medicine, Nashville, Tennessee;* E. Barklis, *Oregon Health & Science University, Portland*

Reverse Transcription/Recombination
Chairpersons: W.-S. Hu, *NCI-Frederick, Maryland;* B. Berkhout, *University of Amsterdam, The Netherlands*

Pathogenesis/Host Factors
Chairpersons: M. Lairmore, *Ohio State University, Columbus;* H. Fan, *University of California, Irvine*

Transcription, RNA Processing, Export, Packaging
Chairpersons: K. Boris-Lawrie, *Ohio State University, Columbus;* J. Dudley, *University of Texas, Austin*

Antivirals
Chairpersons: A. Kaplan, *University of North Carolina, Chapel Hill;* A. Lever, *University of Cambridge, United Kingdom*

M. Laasko, R. Swanstrom

C. Furnes, D. Dobbs

A. Fassati, M. Pizzato

S. Kim, C. Woodward

New York Structural Biology Group

August 6 252 participants

ARRANGED BY **Leemor Joshua-Tor,** Cold Spring Harbor Laboratory
Larry Shapiro, Columbia University College of Physicians & Surgeons

This summer meeting was the sixth 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 nine talks and 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.

Financial support was provided by Amersham Biosciences, Hampton Research, Rigaku MSC and Proterion, Inc.

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*

Session III

Chairpersons: L. Joshua-Tor, *Cold Spring Harbor Laboratory;* B. 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*

Cancer Genetics and Tumor Suppressor Genes

August 18–22 405 participants

ARRANGED BY **Jacqueline Lees,** MIT Center for Cancer Research
Scott Lowe, Cold Spring Harbor Laboratory
Charles Sawyers, University of California, Los Angeles
Charles Sherr, HHMI/St. Jude's Children's Research Hospital

This fourth meeting focused on topics related to cancer genetics and biology. Most of the 400 scientists presented unpublished research through oral or poster presentations. The talks were highlighted by two keynote lectures: Anton Berns discussed recent advances using mice to model human cancer, highlighting their potential utility for identifying new cancer genes and for studying cancer therapeutics. In the second keynote, Stephen Elledge discussed recent advances in elucidating DNA damage responses and their roles in preventing tumorigenesis. An international team of established investigators, whose expertise covered many of the topics highlighted during the meeting, chaired the individual sessions. The first oral session discussed new mouse models, which continue to be an important topic at the meeting. Indeed, many other talks and posters highlighted the use of cancer-prone mice to study gene function and to model human cancer. Other sessions highlighted recent advances in our understanding of DNA damage responses, apoptosis, and cellular senescence, the Rb and p53 tumor suppressor pathways, and signal transduction pathways that are altered in human tumors. This meeting continued to be enthusiastically supported, and the lectures and poster presentations led to extensive discussions and exchanges of information and ideas.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.

G. Evan, L. Attardi, S. Lowe

PROGRAM

Mouse Models
Chairpersons: T. Van Dyke, *University of North Carolina, Chapel Hill;* T. Jacks, *HHMI/Massachusetts Institute of Technology, Cambridge*

Keynote Address: Analysis of the Multifunctionality of BRCA1
D. Livingston, Dana-Farber Cancer Institute, Harvard Medical School

Rb and E2F
Chairpersons: S. Morrison, *HHMI/University of Michigan, Ann Arbor;* J. Lees, *MIT Center for Cancer Research, Cambridge, Massachusetts*

Therapeutics
Chairpersons: C. Thompson, *University of Pennsylvania, Philadelphia;* C.L. Sawyers, *University of California, Los Angeles*

Keynote Address: Cancer Targets in the Ras pathway
Chairperson: Frank McCormick, *University of California, San Francisco*

ARF/p53 Pathway
Chairpersons: G. Evan, *University of California Comprehensive Cancer Center, San Francisco;* K. Vousden, *Beatson Laboratories, Institute for Cancer Research, Glasgow, United Kingdom*

Genome-wide Analysis and Screens
Chairpersons: S.A. Courtneidge, *Van Andel Research Institute, Grand Rapids, Michigan;* M. Van Lohuizen, *University of California, San Francisco*

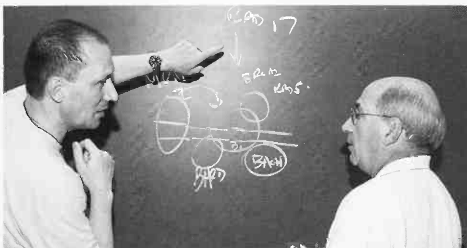
DNA Damage/Chromosomal Instability
Chairpersons: J. Lukas, *Danish Cancer Society, Copenhagen;* T. De Lange, *The Rockefeller University, New York*

Signaling I
Chairpersons: B. Schulman, *St. Jude Children's Research Hospital, Memphis, Tennessee;* E. Holland, *Memorial Sloan-Kettering Cancer Center, New York*

Signaling II
Chairpersons: M.F. Roussel, *St. Jude Children's Research Hospital, Memphis, Tennessee;* L.F. Parada, *University of Texas Southwestern Medical Center, Dallas*

P. Van Sloun, T. Hughes

M. Fried, J. Jenkins, Y. Ito



J. Lukas, D. Livingston

D. Ray, A. Datta

Molecular Genetics of Bacteria and Phages

August 24–29 209 participants

ARRANGED BY **Tina Henkin,** Ohio State University
Catherine Squires, Tufts University School of Medicine
Ryland Young, Texas A&M University

The meeting featured 98 talks arranged in 10 oral presentation sessions, and two poster sessions. Young scientists gave most of the talks, and a senior scientist who presented an introductory overview moderated each session. Topics included ribosomes, translation, and posttranscriptional regulation; bacteriophage development and host interactions; cell division and development; transcriptional regulation; cell surfaces—structure, function, and signaling; host/pathogen interactions; bacterial genomics and proteomics; DNA structure, recombination, and transposition; and stress response, chaperones, and protein degradation.

An interesting theme tying seemingly disparate sessions together was the variety of roles that small or nonencoding RNAs have in influencing gene expression. This theme started with the Nat Sternberg Award winner's lecture (A. Nahvi from the Breaker group at Yale) describing riboswitches, RNAs whose structure is altered when certain small molecules are sensed, leading to a dramatic change in gene expression. Similarly, the Henkin group (Ohio State) demonstrated that the T-box system using binding of charged/uncharged tRNAs to RNA and regulating gene expression works in vitro, providing evidence that no other effectors are needed for the RNA-mediated control. Somewhat unexpectedly, the Losick group at Harvard found that a small RNA inhibits cell lysis in *Bacillus subtilis* and another, RNAIII, was found by collaborating groups from Strasberg, Lyon, and Paris to be involved in regulating expression of pathogenesis genes in *Staphylococcus aureus*. Substantial physiological changes are noted in *Escherichia coli* (Remme group, Estonia) and *C. crescentus* (Keiler group at Penn State) when the small RNA responsible for rescuing stalled ribosomes and tagging incomplete peptides for destruction, tmRNA, is lacking. Further studies by the Weisberg group at NIH of a small RNA encoded by phage HK022 called PUT described how PUT acts in *cis* to alter RNA polymerase into an antitermination-competent form that can transcribe through terminators. In Archaea, Pat Dennis (UBC) described the sequence and structural constraints of small RNAs needed as guides for rRNA modification complexes. The description of new small, noncoding RNAs in *E. coli* (G. Storz group, NIH) suggests that there are more important roles for these RNAs that are not yet fully appreciated. These intriguing reports, and the wide variety of systems giving rise to them, suggest that in future meetings we can expect progress in understanding the contributions to gene expression and cell physiology from a host of different forms of RNAs.

This meeting was funded in part by the National Science Foundation.

C. Squires, O. Amster-Choder

PROGRAM

Ribosomes, Translation, and Posttranscriptional Regulation
Chairperson: R. Green, *Johns Hopkins University, Baltimore, Maryland*

Bacteriophage Development and Host Interactions
Chairperson: M. Gottesman, *Columbia University, New York*

RNA Polymerase: Structure, Function, and Regulation
Chairperson: R. Landick, *University of Wisconsin, Madison*

Cell Division and Development
Chairperson: W. Margolin, *University of Texas Houston Medical School*

Transcriptional Regulation
Chairperson: C. Yanofsky, *Stanford University, California*

Cell Surfaces-Structure, Function, and Signaling
Chairperson: M. Manson, *Texas A&M University, College Station*

Host Pathogen Interactions
Chairperson: H. Shuman, *Columbia University, New York*

Bacterial Genomics and Proteomics
Chairperson: J. Lawrence, *University of Pittsburgh, Pennsylvania*

DNA Structure, Recombination, and Transposition
Chairperson: K. Derbyshire, *New York State Department of Health and University at Albany*

Stress Response, Chaperones, and Protein Degradation
Chairperson: S. Wickner, *National Institutes of Health/NCI, Bethesda, Maryland*

W. Champness, L. Snyder

Z. Pragai, S. Semsey

D. Cully, L. Hernandez, H. Shuman

M. Leibman, J. Roberts

Mouse Molecular Genetics

September 1–5 311 participants

ARRANGED BY **François Guillemot,** National Institute for Medical Research
Hiroshi Hamada, Osaka University
Terry Magnuson, University of North Carolina, Chapel Hill
Janet Rossant, Samuel Lunenfeld Research Institute

Held every other year, this meeting attracted more than 300 researchers from across the globe. Scientists attending the meeting also come from very diverse areas of biology and are united by the common use of mouse mutants in their research. As a result, the meeting covers very diverse areas of biology, in which the contribution of mouse genetics is essential to the progress of knowledge. The sustained interest of all participants throughout the meeting is ensured by the commonality of techniques, as well as by increasing evidence that basic mechanisms are conserved across diverse experimental systems.

The meeting opened with the Annual Rosa Beddington Lecture given by Elisabeth Robertson. The meeting was organized into ten sessions. In a slight departure from the format of previous years, each session was arranged around not two, but three talks of general interests, given by two senior and one more junior invited researchers. Between these more in-depth talks, five to six speakers per session were selected from the abstracts for more concise presentations. The reason for adding one invited speaker to each session was to further increase the visibility and attractiveness of the meeting. The meeting also included the presentation of several hundred posters in two afternoon sessions. The Genetics Society of America sponsored cash prizes for the five best poster presentations as judged by the organizers and other selected PIs. The meeting ended with a farewell to Hiroshi Hamada, who stepped down after 4 years as an organizer of the meeting.

T. Magnuson, D. Stewart

Some of the sessions have shifted in their emphasis this year. The neurobiology session has evolved to represent systems neuroscience, and Linda Buck (2004 Nobel Prize for Medicine) gave an invited lecture in this session on information coding in the olfactory system. The growing emphasis on genetics/genomics and on human disease models, already noted in previous years, has also continued. Aravinda Chakravarti was invited to provide links between mouse and human genetics. Given the increasing importance of the mouse as a model to investigate disease mechanisms, and the sophistication with which diseases can now be modeled and analyzed in the mouse, the organizing committee has decided to emphasize the link between mouse and human genetics.

This meeting was funded in part by the National Cancer Institute, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, the National Human Genome Research Institute, and the National Institute of Neurological Disorders and Stroke, branches of the National Institutes of Health.

PROGRAM

Rose Beddington Lecture: Combinatorial Smad Activities Regulate Nodal-dependent Patterning of the Early Embryo
Elizabeth Robertson, *Harvard University, Cambridge, Massachusetts*

Stem Cells and Differentiation
Chairperson: F. Miller, *Hospital for Sick Children, Toronto, Canada*

Models of Human Disease
Chairperson: R. Lifton, *Yale University School of Medicine, New Haven, Connecticut*

Organogenesis
Chairperson: A. McMahon, *Harvard University, Cambridge, Massachusetts*

Neurobiology
Chairperson: L. Buck, *HHMI/University of Washington, Seattle*

Embryonic Patterning
Chairperson: V. Papaioannou, *Columbia University, New York*

Epigenetics
Chairperson: J. Lee, *Massachusetts General Hospital, Boston*

Genetics and Genomics
Chairperson: L. Flaherty, *Wadsworth Center, Troy, New York*

Signaling
Chairperson: G. Martin, *University of California, San Francisco*

S. Blaess, A. Sudarov

M. Shen, J. Rivera

J. Sanes, T. Grodzicker, G. Martin

Translational Control

September 7–12 475 participants

ARRANGED BY **Alan Hinnebusch,** National Institutes of Health/NICHD
Tatyana Pestova, SUNY Downstate Medical Center
Joel Richter, University of Massachusetts Medical School

This meeting attracted more than 470 participants from around the world and included three keynote speakers, nine platform speakers, and three poster sessions that covered a record 415 abstracts. Several noteworthy reports concerned the mechanism of translation. Contrary to the prevalent model, it was shown that initiation factors eIF1 and eIF3 are not released from 48S complexes after eIF5-stimulated GTP hydrolysis, but rather later when the 60S subunit joins. Evidence was provided for cooperative assembly of eIFs 1, 1A, and ternary complex on 40S ribosomes in vivo, and FRET analysis showed that AUG recognition decreases interaction between eIF1 and eIF1A and weakens eIF1 binding to the 40S ribosome. The NMR structure of eIF2α revealed an unexpected similarity with eEF1B, the GEF for elongation factor eEF1A. Furthermore, it was shown that eIF2α kinases and eIF2B (the GEF for eIF2) recognize overlapping surfaces in eIF2α. Regarding translation termination, two reports indicated that RRF does not function as a tRNA mimic in catalyzing ribosome disassembly, based on its mode of binding to the 50S subunit and its independence of the translocation activity of EF-G. It was shown that eRF3 is not involved in recycling eRF1 but participates in the decoding process or peptide chain release.

T. Pestova, J. Richter, A. Hinnebusch

The cryo-EM structure of the CrPV internal ribosome entry (IRES) bound to the 80S ribosome revealed specific contacts with the A, P, and E sites and suggested that it functions as RNA-based translation factor that "pushes" the ribosome into an elongation mode. A polypyrimidine-rich motif that binds PTB was shown to be sufficient to direct PTB-dependent IRES when present as a double-stranded stem.

On the topic of translational control, it was shown that translational regulation of mammalian ATF4 mRNA via eIF2α phosphorylation closely resembles the reinitiation mechanism involving uORFs described for yeast GCN4. Phosphorylation of eIF2α also was shown to activate NF-κB in cells experiencing ER stress, and to increase resistance to hypoxic stress in tumor cells. Stress activation of the cytostatic protein kinase Pak2 leads to phosphorylation of eIF4G and the inhibition of translation in vivo. It was reported that phosphorylation of the Glu/Pro-tRNA synthetase and the ribosomal protein L13a releases them from the multisynthetase complex and the 60S ribosomal subunit to collaborate in a complex that binds a stem-loop in the 3′UTR of ceruloplasmin mRNA to repress translation. In a similar vein, ribosomal protein L30 was implicated as part of the SECIS-binding element in the 3′UTR of mRNAs that incorporate selenocysteine-tRNA at UGA codons. Evidence was presented that base pairing of a liver-specific microRNA (miR122) to the 5′UTR of hepatitis C virus is required for virus replication, and it was found that interferon induces the RNA editing enzyme ADAR, which specifically deaminates HCV RNA and blocks virus replication. The maskin model for mRNA-specific translational control by proteins that bind to the 3′UTR and disrupt the eIF4E-eIF4G interaction at the cap has emerged as a major paradigm for gene-specific translational control in metazoan development. For example, *Drosophila* embryos contain two new maskin-like molecules: Cup, whose control of mRNA-specific translation is necessary for oocyte and embryo development, and Midline, whose similar mRNA-specific translation is required for axon pathfinding in the developing central nervous system.

In the arena of mRNA decay, evidence was presented that a yeast ribosome at a premature stop codon is inefficiently released relative to a natural stop codon and triggers nonsense-mediated decay because of its separation from mRNP complexes in the 3′UTR. mRNA decay and NMD were found to occur within P-bodies in yeast. The crystal structure of the DcpS scavenger decapping enzyme was shown to be an asymmetric dimer containing two distinct active sites bound by two cap molecules. Finally, it was shown that tyrosine

phosphorylation of the *Xenopus* polysome-associated endoribonuclease PMR1 targets it to the substrate mRNA in polysomes.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development, branches of the National Institutes of Health.

PROGRAM

Keynote Addresses: Decoding of mRNA by the Ribosome
V. Ramakrishnan, *MRC Laboratory of Molecular Biology, University of Cambridge, United Kindom*

Intracellular Signaling from the Endoplasmic Reticulum to the Nucleus
P. Walter, *HHMI/University of California, San Francisco*

RNA Regulation in the Germ Line
J. Kimble, *HHMI/University of Wisconsin, Madison*

Initiation Factors: Structure and Function
Chairperson: R. Jackson, *University of Cambridge, United Kingdom*

Viral Regulatory Strategies
Chairperson: W.A. Miller, *Iowa State University, Ames*

Translation Mechanisms I
Chairperson: G. Wagner, *Harvard Medical School, Boston, Massachusetts*

Translation Mechanisms II
Chairperson: Y. Nakamura, *University of Tokyo, Japan*

Developmental Regulation
Chairperson: E. Gavis, *Princeton University, New Jersey*

Regulation of Factors
Chairperson: J. Traugh, *University of California, Riverside*

mRNA Turnover
Chairperson: M. Kiledjian, *Rutgers University, New Brunswick, New Jersey*

Regulatory Elements in mRNA
Chairperson: D. Gallie, *University of California, Riverside*

Internal Initiation
A. Willis, *Leicester University, United Kingdom*

T. Michael, L. Ryabova

A. Jacobson, J. Pelletier

K. Sherrill, S. Timmerman

CSHL/WELLCOME TRUST CONFERENCE
Functional Genomics of Host-Pathogen Interactions

September 9–12 105 participants

ARRANGED BY **Matthew Berriman,** The Wellcome Trust Sanger Institute
John Boothroyd, Stanford University
Julian Parkhill, The Wellcome Trust Sanger Institute
George Weinstock, Baylor College of Medicine

The first meeting on functional genomics of host-pathogen interactions was held at the Wellcome Trust Genome Campus in Hinxton, United Kingdom, and was a joint project of CSHL and the Wellcome Trust. The intention of the meeting was to bring together scientists working on host-pathogen interactions in a wide variety of systems, both eukaryotic and prokaryotic, to explore potential areas of overlap and cross-talk between these sometimes disparate fields. To support this intention, the sessions of the meeting were not themed, and each contained an eclectic mix of invited and offered talks on a wide variety of systems. Subjects covered included bacterial pathogens of man and animals; bacterial symbionts of parasitic worms and insect vectors; parasite interactions with mammalian (and plant) hosts and insect vectors; fungal pathogens of plants, animals, and humans; and viral pathogens of humans and animals. The limited size and broad spread of the meeting allowed for a great deal of productive interactions among the participants outside the formal sessions, and the meeting appeared to be very well received by the attendees.

PROGRAM

Session 1
Chairpersons: R. Maizels, *University of Edinburgh, United Kingdom;* J. Barnwell, *Centers for Disease Control & Prevention, Atlanta, Georgia*

Session 2
Chairpersons: J. Ewbank, *University of Marseilles, France;* G. Dougan, *The Sanger Institute, Cambridge, United Kingdom*

Session 3
Chairpersons: F. Kafatos, *EMBL, Heidelberg, Germany;* B.J. Hinnebusch, *National Institutes of Health/NIAID, Hamilton, Montana*

Session 5
Chairpersons: D. Smith, *Imperial College, London, United Kingdom;* M. Hook, *Texas A&M University Health Science Center, Houston*

Session 6
Chairpersons: C. d'Enfert, *Institut Pasteur, Paris, France;* A. Tait, *University of Glasgow, United Kingdom*

Keynote Address: Host-pathogen Encounters: Multiple Genomes at Play
D. Relman, *Stanford University, Palo Alto, California*

Session 7
Chairpersons: A.B. van't Wout, *University of Washington, Seattle;* S. Aksoy, *Yale University School of Public Health, New Haven, Connecticut*

Wellcome Trust Sanger Institute, Hinxton, UK

Axon Guidance and Neural Plasticity

September 18–22 422 participants

ARRANGED BY **Cori Bargmann,** HHMI/University of California, San Francisco
Mu-Ming Poo, University of California, San Diego
Joshua R. Sanes, Harvard University

The remarkable information processing performed by the human brain is made possible by the intricate connections between nerve cells, or neurons. The magnitude of the task involved in wiring the nervous system is staggering. In adult humans, billions of neurons each make connections with, on average, more than 1000 target cells. A stereotyped initial pattern of connections is modified by experience and learning to create sophisticated memories and behaviors. How is the pattern of neuronal wiring generated during embryogenesis with the necessary precision and reliability? Neuronal connections form when each developing neuron sends out an axon, tipped by a growth cone, which migrates through the embryonic environment to its synaptic targets, guided by attractive and repulsive proteins that instruct it to migrate in particular directions. Once in the appropriate target regions, axons must seek out particular target cells with which to form synaptic connections. These connections are then further refined, through the making and breaking of synaptic contacts, under the control of specific patterns of electrical activity in the neurons and targets, until a highly tuned circuit is established.

J. Sanes, C. Bargmann

In the past decade, our understanding of the mechanisms that control axon growth and guidance, synaptogenesis, and the remodeling of neural circuits during development has progressed rapidly from phenomenology to the identification of specific molecular control mechanisms. Progress has been assisted by the finding that these mechanisms are highly conserved across evolution, so that both biochemical approaches in vertebrates and genetic approaches in invertebrates (and increasingly, in vertebrates as well) have led to mutually reinforcing discoveries that have helped fuel further advances.

As the pace of discovery has quickened, the field has grown enormously, making it more difficult for scientists to keep abreast of new developments. To help facilitate communication in the field, a biennial CSHL conference series on *Axon Guidance and Developmental Plasticity of the Nervous System* was initiated in 1998. This year, the fourth of these meetings involved sessions devoted to particular problems in the assembly of the nervous system, with speakers chosen from among the participants submitting abstracts by session chairs who are leaders in the field. Other abstracts were presented as posters.

As for the first three meetings, the response of the field to this conference was one of overwhelming enthusiasm. Of the 422 registrants, 288 submitted abstracts; 62 abstracts were selected for talks, in eight sessions. Senior researchers, starting assistant professors, postdoctoral fellows, and graduate students were well represented as speakers and participants. All of the major areas of research in the field were covered, as were all of the major approaches (cellular, physiological, anatomical, biochemical, and genetic). In addition, there were two keynote addresses: Dr. Joe LeDoux (New York University) focused on neural plasticity and the basis of emotional behavior, and Dr. Atsushi Miyawaki (RIKEN) focused on the use of fluorescent reporter technologies for measuring intracellular signaling events with high spatial and temporal precision. The meeting provided an important clearinghouse for ideas and approaches and helped scientists in the field get the most up-to-date information, as well as enabling them to meet, to network, and to establish collaborations. Based on the uniformly enthusiastic comments of the participants, the intensity of the oral and poster sessions, and the large crowds that stayed up late every night at the bar to discuss science, the meeting was a great success.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health.

PROGRAM

Axon Guidance: Molecules
Chairpersons: L. Zipursky, *HHMI/University of California, Los Angeles;* C. Holt, *University of Cambridge, United Kingdom*

Synaptic Plasticity, Arborization, and Circuits
Chairpersons: L. Katz, *HHMI/Duke University Medical Center, Durham, North Carolina;* T. Hensch, *RIKEN Brain Science Institute, Saitama, Japan*

Special Lecture: Emotional Synapses
J. LeDoux, *New York University*

Regeneration and Remodeling
Chairpersons: S. Strittmatter, *Yale University, New Haven, Connecticut;* O. Hobert, *Columbia University College of Physicians & Surgeons, New York*

Cellular and Molecular Maps
Chairpersons: D. O'Leary, *Salk Institute, La Jolla, California;* C. Mason, *Columbia University, New York*

Cell Biology
Chairpersons: K. Svoboda, *HHMI/Cold Spring Harbor Laboratory;* A. Ghosh, *University of California, San Diego*

Synapse Formation
Chairpersons: J. Lichtman, *Washington University, St. Louis, Missouri;* G. Turrigiano, *Brandeis University, Waltham, Massachusetts*

Special Lecture
A. Miyawaki, *RIKEN Brain Science Institute*

Dendrites and Diversity
Chairpersons: H. Cline, *Cold Spring Harbor Laboratory;* T. Bonhoeffer, *Max-Planck Institute for Neurobiology, Martinsried, Germany*

Signaling
Chairperson: F. Gertler, *Massachusetts Institute of Technology, Cambridge*

T. Hensch, K. Svoboda

F. Rathjen, E. Stoeckli

K. Brose, J. Huang

G. Little, A. Rünker, S. Miller, J. Dolan

CSHL/WELLCOME TRUST CONFERENCE
Genome Informatics

September 22–26 285 participants

ARRANGED BY **Ewan Birney,** European Bioinformatics Institute
Suzanna Lewis, University of California, Berkeley
Lincoln Stein, Cold Spring Harbor Laboratory

This third conference on *Genome Informatics* focused on large-scale genome annotation and utilization. The rationale behind organizing a joint conference in Europe derives from the increasingly competitive academic conference market, with many institutions in the United States and Europe openly planning to emulate the success of the CSHL meetings model. The conference was 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 Centre, the European Bioinformatics Institute, and the HGMP–MRC Resource Centre. The conference followed a format similar to traditional CSHL meetings, in that the majority of oral presentations were drawn from openly submitted abstracts.

The explosion of biological data requires a concomitant increase in the scale and sophistication of information technology, ranging from the storage of data and their associated data models, to the design of effective algorithms to uncover nonobvious aspects of these datasets, 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. Meeting sessions included comparative genomics, genome-wide analyses, genome databases, and genomic pipe lines.

PROGRAM

Large-scale Genomics I
Chairpersons: M. van Baren, *Washington University, St. Louis, Missouri;* S. Searle, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Genome Databases
Chairperson: C.J. Bult, *Jackson Laboratory, Bar Harbor, Maine*

Comparative Genomics
Chairperson: E. Sonnhammer, *Karolinska Institutet, Stockholm, Sweden*

Transcription and RNA Analysis
Chairperson: A. Krogh, *University of Copenhagen, Denmark*

Interactions and Networks
Chairperson: P. Carninci, *RIKEN Genomic Science Laboratory, Wako, Japan*

Functional Annotation and Proteomics
Chairpersons: J. Swedlow, *University of Dundee, United Kingdom;* A. Pandey, *Johns Hopkins University, Baltimore, Maryland*

Large-scale Genomics II
Chairpersons: M. van Baren, *Washington University, St. Louis, Missouri;* S. Searle, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Keynote Address
M. Scott, *Stanford University School of Medicine*

Genomic Pipelines
Chairperson: D. Rokhsar, *Joint Genome Institute, Walnut Creek, California*

Wellcome Trust Sanger Institute, Hinxton, UK

Dynamic Organization of Nuclear Function

September 29–October 3 254 participants

ARRANGED BY **Wendy Bickmore,** MRC Human Genetics Unit
Michael P. Rout, The Rockefeller University
David Spector, Cold Spring Harbor Laboratory

This meeting focused on the relationships between nuclear structure and function. The opening session highlighted defects in nuclear structure that occur in human disease, from both the perspective of nuclear proteins and DNA sequence. Mutations in lamin A are found in an increasing number of diseases (laminopathies). C. Stewart, B. Burke, and J. Lammerding showed that lamin A mutations weaken the mechanical strength of the nucleus and affect signaling and gene expression. Compelling evidence for a connection between the cytoskeleton and the nucleus was presented. Most intruigingly, the lamin-A mutations found in a form of progeria were suggested to profoundly alter chromatin structure (R. Goldman) and the nuclear abnormalities in these cells could be reverted through modulating splicing of lamin-A mRNA (P. Scaffidi). The roles of D4Z4 repeats, the FRG1P protein, and nuclear organization in another form of muscular dystrophy (FSHD) remain an enigma (S. van Koningsbruggen and J. Lawrence).

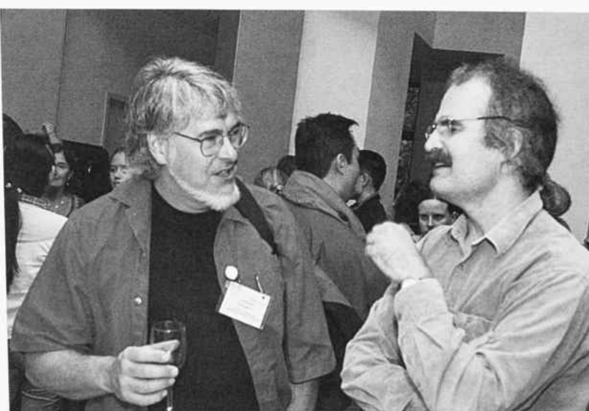
W. Bickmore, D. Spector, M. Rout

The session on chromosomes and the cell cycle illustrated the power of conditional mutagenesis, and RNAi, in dissecting the function of proteins involved in chromosome segregation (W. Earnshaw, T. Fukagawa, M. Mazumdar). Complementing this, W. Earnshaw (using mass spectrometry) and H. Funabiki (using an expression screen in *Xenopus*), both identified a novel chromosome passenger protein (Borealin/DasraB) involved in kinetochore-microtubule interactions and spindle stability.

The role of nuclear organization in regulating gene expression is becoming more widely recognized. A central theme of the session on chromosomes and gene regulation was the importance of concentrating genes and proteins at specific sites within the nucleus, to enhance the efficiency of either transcription or gene silencing. A. Taddei showed how pathways for silencing and perinuclear anchoring can be dissected in yeast and revealed the anchoring role of Esc1, an acidic coiled-coil protein of the inner nuclear membrane, which may be a functional homolog of lamin. Nuclear colocalization of loci from the Antennapedia and bithorax complexes at concentrations of polycomb protein were shown in *Drosophila* nuclei (G. Cavalli). On the other hand, C. Osborne presented evidence to suggest that transcriptionally active genes colocalize in the nucleus, at concentrations of RNA polymerase II (transcription factories). W. Bickmore used a biophysical approach to suggest that changes in the structure of the basic 30-nm chromatin fiber affect chromatin condensation in the nucleus and the subnuclear location of loci relative to chromosome territories. The nuclear organization of chromatin proteins and transcription factors, relative to heterochromatin and other nuclear structures, was also described (W. de Laat, J. Mateos-Langerak, F. Schaufele).

The session on RNA processing and dynamics touched on a variety of RNA species and processing steps. Two talks examined the dynamics of a population of nuclear poly(A)$^+$ RNA that is not nascent and is found in nuclear speckles. Using different approaches, one study showed that this RNA population is highly dynamic and exchanges with the nucleoplasmic pool in an ATP-independent manner (J. Ritland-Politz), whereas the second study indicated that 50% of the mRNA present in speckles is immobile (U. Schmidt). An examination of the rate of transcription in living cells indicated that RNA synthesis occurs at an average rate of 1.5 kb/min; however, the rate varied along the template (X. Darzacq). A study examining the entry of pre-mRNA processing factors into daughter nuclei after mitosis showed that hypophosphorylated SR proteins were transiently associated with the periphery of nucleolar organizing regions; the duration of this interaction

M. Seguar-Valdez, L. Jimenez-Garcia



W. Earnshaw, Y. Lazebnik

could be extended by inhibiting the synthesis of pre-mRNA transcripts (P.A. Bubulya). snoRNAs form a series of distinct RNP complexes that are involved in different RNA-processing events. U.T. Meier examined the assembly of such complexes and found that NAP57 and NOP10 provide an initial platform for subsequent RNP assembly.

For many years, the mechanism of the nucleocytoplasmic exchange of macromolecules has been mysterious. Recent advances in our understanding of the nuclear pore complex (NPC)-mediated transport process were introduced by S. Wente, who then described work in which strains carrying various deletions in the NPC-associated karyopherin docking sites were assessed for their ability to support various types of nucleocytoplasmic transport. Her data favor a model in which diffusion-restricted passage across the NPC is catalyzed by the binding of karyopherins to specific docking sites. The structure of the NPC has also been investigated using cryo-electron tomography techniques on isolated, transport-competent nuclei (O. Medalia). New details of the NPC's structure were revealed, which agreed well with a map of the NPC generated by synthesizing various biochemical and structural data (M. Rout). L. Gerace used an ingenious assay to investigate how proteins are targeted to the inner nuclear membrane and proposed as a result that this process is diffusion-mediated and energy-dependent. Connections between the processes of nuclear transport and nuclear assembly were introduced by D. Forbes, who described how one karyopherin, importin-β, behaved as an inhibitor of the first stages of nuclear reassembly after mitosis. The protein Pom121 was shown to be crucial to this assembly process (W. Antonin), and titin (a huge protein associated with muscle) was shown to be important for the structure of nuclear envelopes in *Caenorhabditis elegans* (M. Zastrow). Indeed, C. Brown indicated that the general organization of the nuclear periphery is crucial for the correct expression of genes, as genes in *Saccharomyces* can dynamically localize closer to the NPC upon their activation.

mRNA can only be translated if it leaves the nucleus. K. Prasanth described a noncoding RNA that is retained in the nucleus and that localizes to paraspeckles. Most intriguingly, this RNA is A-to-I edited, and it was suggested that this functions to retain this RNA in the nucleus. Normal mRNAs are exported from the nucleus as mRNP particles. Exciting evidence was presented that, in yeast, MLP proteins attached to the nuclear pore act to check the quality of mRNPs for nuclear export (F. Stutz, M. Fasken). Other unexpected connections were made between the nuclear pore and DNA repair (V. Doye).

There was significant progress on understanding the structure, composition, and function of nuclear bodies. J. Gall described the unexpected finding that Cajal bodies, in the germinal vesicle of *Xenopus*, contain acetylated histone H4. He also showed that in *Drosophila* Cajal bodies localize at histone genes, as has been shown in mammalian cells. Developing this theme, G. Matera showed that mammalian Cajal bodies localize to a BAC transgene that contains inducible U2 snRNA genes and that this interaction is dependent on RNA. Cajal bodies are known to be involved in the biogenesis of snRNAs and snoRNAs. M. Terns presented evidence that they may also be involved in the biogenesis of telomerase RNA. The function of PML bodies remain somewhat of an enigma. H. de Thé gave insight into the complexity of PML function by explaining

the different functions attributed to these bodies and the subnuclear localization of PML protein isoforms.

Progress in understanding nuclear structure and function has depended on the development of new technologies and experimental approaches. The session on emerging technologies promises even more to come. D. Bazzett Jones showed how energy-filtered transmission electron microscopy can be used to investigate the distribution of proteins and nucleic acid at PML bodies. The resolution limit of the light microscope is also being improved. J. Sedat presented provocative new data on the structure of metaphase chromosomes using method-saturated structured illumination microscopy. C. Cremer presented examples of applications using 4Pi-, spatially modulated illumination, and spectral precision distance microscopy. Finally, proteomics is having an important role in defining not only the components of nuclear bodies, but also their dynamic behavior. A. Lamond showed how heavy isotope labeling combined with mass spectrometry can elucidate changes in nucleolar composition in response to metabolic stimuli and the cell cycle

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided support for this meeting.

PROGRAM

Nuclear Structure and Disease
Chairperson: C.L. Stewart, *National Cancer Institute, Frederick, Maryland*

Chromosomes and the Cell Cycle
Chairperson: W.C. Earnshaw, *Wellcome Trust Centre for Cell Biology, Edinburgh, United Kingdom*

Emerging Technologies to Assess Nuclear Function
Chairperson: J. Sedat, *University of California, San Francisco*

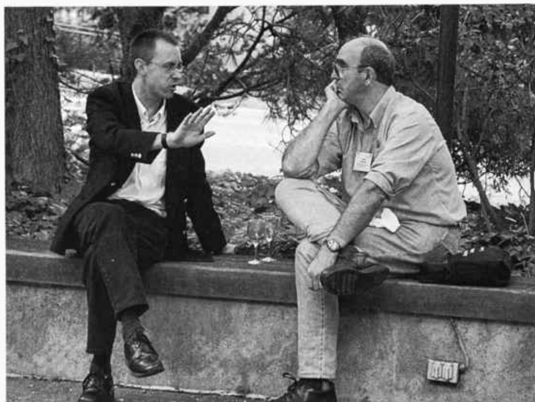
The Nuclear Periphery
Chairperson: S.R. Wente, *Vanderbilt University Medical Center, Nashville, Tennessee*

RNA Processing and Dynamics
Chairperson: T. Pederson, *University of Massachusetts Medical School, Worcester*

Chromosome Organization and Gene Regulation
Chairperson: A.G. Fisher, *Medical Research Council, London, United Kingdom*

Transcription and mRNA Export
Chairperson: W. Bickmore, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

Nuclear Bodies
Chairperson: H. de Thé, *CNRS Hôpital St. Louis, Paris, France*



I. Uings, S. Hauschka

Molecular Genetics of Aging

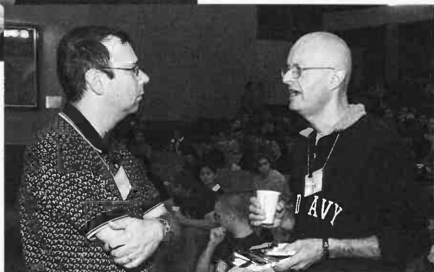
October 6–10 224 participants

ARRANGED BY **Ronald DePinho,** Dana Farber Cancer Institute
Leonard Guarente, Massachusetts Institute of Technology
Linda Partridge, University College London

Aging is a process that leads to many degenerative changes and death. Recently, specific processes closely associated with aging, as well as genes that may regulate aging have been identified. This meeting focused on these advances and provided a forum for discussion of many theories—old and new. Two regulators of aging were featured in several sessions, one involving signaling pathways by insulin-like molecules, and the other a novel class of NAD-dependent deacetylases, called SIR2 proteins. Altering these pathways was shown to extend lifespan in yeast, *Caenorhabditis elegans*, *Drosophila*, and mice. A session was also dedicated to stem cell biology and transcription profiling of aging cells. Two other sessions related to telomere biology and cellular senescence. Premature aging syndromes and diseases of aging were also featured. Finally, recent findings relating aging to metabolism and oxidative stress closed the conference. It was clear that very rapid progress is being made to understand aging and its regulation at a molecular level.

This meeting was funded in part by the National Institute on Aging, 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 support for this conference.

J. Tower, C. Kenyon, L. Partridge



L. Miller, L. Guarente

Model Systems I
Chairpersons: G. Ruvkun, *Massachusetts General Hospital, Boston;* S. Helfand, *University of Connecticut Health Center, Farmington*

Stem Cells and Genomics
Chairpersons: S.K. Kim, *Stanford University Medical Center, California;* R. McKay, *National Institutes of Health/NINDS, Bethesda, Maryland*

Cancer Teleomeres, Etc.
Chairpersons: V. Lundblad, *Baylor College of Medicine, Houston, Texas;* J. Hoeijmakers, *Erasmus University, Rotterdam, The Netherlands*

Cell Senescence
Chairpersons: J. Campisi, *Lawrence Berkeley National Laboratory, California;* S. Korsmeyer, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

Model Systems II
Chairpersons: L. Partridge, *University College London, United Kingdom;* C. Kenyon, *University of California, San Francisco*

Mice Altered Aging
Chairpersons: A. Bartke, *Southern Illinois University School of Medicine, Springfield;* R. Weindruch, *University of Wisconsin, Madison*

Mouse Models Disease
Chairpersons: D. Price, *Johns Hopkins University, Baltimore, Maryland;* B. Spiegelman, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Biochemistry and Pharmacology
Chairpersons: A. Richardson, *University of Texas Health Science Center, San Antonio;* D.A. Sinclair, *Harvard Medical School, Boston, Massachusetts*

M. Zhang, A. Samuelson, G. Ruvkun

D. Smith, T. Sasaki

Germ Cells

October 13–17 227 participants

ARRANGED BY **Blanche Capel,** Duke University Medical Center
Judith Kimble, HHMI/University of Wisconsin

Germ cells are the only cells in the body with the potential for immortality. During the past few years, we have learned that many of the fundamental molecular mechanisms required for establishment and function of germ cells have been conserved during evolution. This meeting was organized with the goal of bringing together researchers who study germ-line development in many organisms, including humans, mice, nematodes, and flies. The big questions addressed were: How are germ-line stem cells specified? How are germ cells regulated to continue in the mitotic cell cycle or transition to the meiotic cell cycle? Do germ cells differ from embryonic stem cells? An important and far-reaching concern was the importance of regulation at the level of DNA and chromatin, as well as regulation at the level of RNA and translation. Both DNA and RNA controls have profound and specific effects on germ-line development. The analyses of these controls in germ cells have led to the discovery of conserved regulators that control germ cells throughout the animal kingdom. Given our growing understanding of molecular mechanisms that control germ-line development and maturation in model organisms, the implications of these findings for stem cell biology and human reproduction were actively discussed. The next *Germ Cells* meeting will be held in the fall of 2006 with Susan Strome and Azim Surani as the coorganizers.

This meeting was funded in part by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health; the March of Dimes; and the Lalor Foundation.

B. Capel, S. Malki

J. Kimble, R. Wharton

Germ-line Genome and Its Regulation
Chairpersons: M. Fuller, *Stanford University School of Medicine, California;* S. Strome, *Indiana University, Bloomington*

RNA Regulation of the Germ Line
Chairpersons: R. Braun, *University of Washington School of Medicine, Seattle;* E. Goodwin, *University of Wisconsin, Madison*

Sexual Dimorphism and Its Control
Chairpersons: S. DiNardo, *University of Pennsylvania School of Medicine, Philadelphia;* R. Lovell-Badge, *MRC National Institute of Medical Research, London, United Kingdom*

Mitosis vs. Meiosis
Chairpersons: D. Page, *HHMI/Massachusetts Institute of Technology, Cambridge:* A. Villeneuve, *Stanford University School of Medicine, California*

Signaling and the Germ Line
Chairpersons: J. Eppig, *Jackson Laboratory, Bar Harbor, Maine;* E. Matunis, *Johns Hopkins University, Baltimore, Maryland*

Germ Cell Transits and Transitions
Chairpersons: R. Lehmann, *Skirball Institute, New York University School of Medicine;* C. Wylie, *Children's Hospital Research Foundation, Cincinnati, Ohio*

Stem Cells
Chairpersons: A. McLaren, *University of Cambridge, United Kingdom;* H. Scholer, *University of Pennsylvania, Kennett Square*

Germ Cell Specification
Chairpersons: G. Seydoux, *Johns Hopkins University School of Medicine, Baltimore, Maryland;* A. Surani, *Wellcome/CRC Institute, Cambridge, United Kingdom*

A. Toure, P. Burgoyne

T. Sunanaga, K. Kawamura, A. Mahowald, Y. Niki

Identification of Functional Elements in Mammalian Genomes

November 11–13 239 participants

ARRANGED BY **Eric Green,** National Human Genome Research Institute/NIH
Webb Miller, Pennsylvania State University
Michael Q. Zhang, Cold Spring Harbor Laboratory

The third CSHL Workshop on Computational Biology focused on *Identification of Functional Elements in Mammalian Genomes* and was preceded by a two-day gathering of the ENCODE (Encyclopedia of DNA Elements) project. This is a public consortium launched by the National Human Genome Research Institute in 2003, which attracted over 100 participants, aimed eventually at providing a fully annotated reference sequence of the human genome. The main workshop attracted almost 240 participants and featured the sessions listed below. Each session began with an introduction by an expert in the field, which provided a useful overview for seasoned and junior scientists alike, and was ably summarized by Rick Myers.

E. Green, R. Myers, H. Sussman

M. Pollock, F. Collins

PROGRAM

Protein-coding Sequences
Chairperson: E. Birney, *European Bioinformatics Institute, Hinxton, United Kingdom*

Elements That Regulate Gene Transcription
Chairperson: M. Green, *HHMI/University of Massachusetts Medical School, Worcester*

RNA Transcripts
Chairperson: C. Burge, *Massachusetts Institute of Technology, Cambridge*

Other Genomic Functional Elements
Chairperson: A. Feinberg, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Future Horizons
Chairperson: D. Haussler, *HHMI/University of California, Santa Cruz*

Meeting Summary
R. Myers, *Stanford University, Palo Alto, California*

J. Ecker, F. Bushman

E. Feingold, P. Good

W. Miller, P. Boutros

Pharmacogenomics

November 18–21 243 participants

ARRANGED BY **David Bentley,** The Wellcome Trust Sanger Institute
J. Steven Leeder, Children's Mercy Hospital
Munir Pirmohamed, University of Liverpool
Richard M. Weinshilboum, Mayo Medical School

This second annual meeting was a joint project of CSHL and the Wellcome Trust and was funded in part by the National Institute of General Medical Sciences, a branch of the National Institutes of Health. The primary objective of the meeting was to stimulate interest from a broadly based constituency representing basic scientists and clinical investigators from both academic and corporate environments by focusing on diverse issues related to the application of genomic approaches to investigations of variability in drug response in humans. The meeting opened with a session presenting pharmacogenomics from the perspectives of the pharmaceutical industry and academia, as well as an example of the potential promise of personalized medicine. Major subject areas for the meeting included pharmacogenomics of drug biotransformation and transport, cancer pharmacogenomics, cardiopulmonary pharmacogenomics, and neuropsychiatric pharmacogenomics. Presentations highlighting economic and social issues related to the impact of pharmacogenomics on clinical practice stimulated considerable audience participation, and issues related to special populations, such as children and ethnic minorities, were addressed in a panel discussion. Perusal of the meeting content, both oral and poster presentations, revealed a movement of experimental approaches away from a focus on single-nucleotide polymorphisms in single genes to more broadly based strategies involving haplotypes of multiple genes and gene pathways.

S. Leeder

The meeting was attended by 243 registrants from 18 countries, compared to 140 attendees from 15 countries at the previous meeting. Women represented 35% of attendees, whereas Ph.D. students and postdoctoral fellows constituted 23%. Feedback from the meeting has been positive and future efforts will directed toward two main goals: (1) increasing representation at the meeting from the genetics and genomics community, and (2) providing more opportunities for trainees and junior faculty members to present their work in oral platform sessions. This meeting provides an outstanding forum to explore the impact of genomics on the treatment of human disease to describe the science underlying pharmacogenomics and to discuss the accompanying benefits and challenges relevant to the implementation of pharmacogenomics in both drug development and clinical settings.

A. Marianki, S. Koukouritaki

This meeting was funded in part by the National Institutes of General Medical Sciences, a branch of the National Institutes of Health.

PROGRAM

Opening Session

Chairpersons: R. Weinshilboum, *Mayo Medical School, Rochester, Minnesota;* R. Long, *National Institutes of Health/NIGMS, Bethesda, Maryland*

Pharmacogenomics of Metabolism and Transport

Chairpersons: K. Giacomini, *University of California, San Francisco;* D. Flockhart, *Indiana University School of Medicine, Indianapolis*

Cancer Pharmacogenomics

Chairpersons: C.R. Wolf, *University of Dundee, United Kingdom;* M. Ratain, *University of Chicago, Illinois*

Cardiopulmonary Pharmacogenomics

Chairpersons: A. Rane, *Karolinska Institutet, Stockholm, Sweden;* J. Johnson, *University of Florida, Gainesville*

Remarks

J.D. Watson, *Cold Spring Harbor Laboratory*

Neuropsychiatric Pharmacogenomics

Chairpersons: J.S. Leeder, *Children's Mercy Hospital, Kansas City, Missouri;* M. Pirmohamed, *University of Liverpool, United Kingdom*

Panel Session: Special Populations and Pharmacogenomics, Future Directions

Chairpersons: A. Breckenridge, *Medicines and Healthcare Products Regulatory Agency, London, United Kingdom;* D. Bentley, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

K. Philips, C. Stephens, C. Ross

A. Daly, A. Alfirevic, J. Foster

CSHL Winter Biotechnology Conference: Drug Discovery in Neurodegenerative Diseases

December 2–5 122 participants

ARRANGED BY **Sam Gandy,** Farber Institute for Neurosciences at Thomas Jefferson University
Virginia M.-Y. Lee, University of Pennsylvania School of Medicine
Marcy MacDonald, Massachusetts General Hospital/Harvard Medical School

As many as one half of people aged 65 years or older will develop debilitating degenerative disease of the central nervous system, usually characterized by a decade or more of dependent living, accompanied by progressive failure of cognitive function and/or coordinated movement. Although these illnesses appear most commonly in the absence of obvious heredibility or identifiable genetic mutations, it has been possible during the past 20 years to discover risk-modifying DNA changes in some examples and predictable causative changes in others. From these findings, transgenic technology has rapidly led to the development of mouse, fruit fly, and nematode model systems that partially recapitulate the clinical abnormalities of the human diseases, as well as some of the hallmark molecular and morphological pathology of the conditions.

I. Gozes, V.M.-Y. Lee

Rational biochemical and cell-based screens have generated lead compounds that show promise in the living animal models. Most importantly, the animal models have enabled discovery of entirely unanticipated therapeutic strategies (such as amyloid-β immunotherapy). In December 1999, some of these rationally discovered compounds and unexpected immunotherapies entered Phase I clinical trials. The progress of compounds and rational strategies from the animal model to the human clinical trial; the design, results, and conclusions of trials; and the return to the animal model with questions raised during human trials are the areas of particular emphasis for these biannual Winter Biotechnology Meetings instituted in December 2000 with the explicit goal of facilitating the translation of "breakthrough" science into effective medicines.

For the 2004 meeting, seven 3- or 4-hour platform sessions were organized around common technological themes. Chairpersons, invited speakers, and speakers selected from submitted abstracts were drawn from the academic and pharma sectors. Although the discussion of new, unpublished data was emphasized, the group was tolerant of a wide range of intellectual property conventions. Poster presentations were also encouraged: Posters were displayed for an extended period during the meeting, and poster viewing was especially encouraged during the cocktail hours before the evening meals.

Topics included protein aggregation inhibitors, protein processing enzymology and pharmacology, immunotherapy, design and interpretation of genomic analyses in mammalian and lower systems, bioinformatics, mouse, nematode, and fly models of neurological disease, mechanisms of neurodegeneration, metabolic and hormonal influences on disease protein metabolism in cell culture and living animal models, as well as in human clinical trials, and the importance of applying high-throughput screening strategies to academic efforts ("chemical genomics"), as well as its typical use in drug discovery. Diseases considered included Alzheimer's, Huntington's, spinocerebellar atrophies, ALS, prion diseases, Parkinson's, tauopathies, and synucleinopathies.

Pharmacology of Proteolysis
Chairpersons: S. Gandy, *Thomas Jefferson University, Philadelphia, Pennsylvania;* M. MacDonald, *Massachusetts General Hospital, Charlestown*

The Way Forward: NIH Roadmap and Innovative HTS Strategies
Chairperson: V. Man-Yee Lee, *University of Pennsylvania, Philadelphia*

Intracellular Aggregation I
Chairperson: M. MacDonald, *Massachusetts General Hospital, Charlestown*

Intracellular Aggregation II
Chairperson: V. Man-Yee Lee, *University of Pennsylvania, Philadelphia*

Intracellular Aggregation III
Chairperson: M. MacDonald, *Massachusetts General Hospital, Charlestown*

Extracellular Aggregates I
Chairperson: S. Gandy, *Thomas Jefferson University, Philadelphia, Pennsylvania*

Extracellular Aggregates II
Chairperson: S. Gandy, *Thomas Jefferson University, Philadelphia, Pennsylvania*

Intracellular Aggregation IV
Chairpersons: M. MacDonald, *Massachusetts General Hospital, Charlestown;* V. Man-Yee Lee, *University of Pennsylvania, Philadelphia*

M. Sekuma, C. Mizumarau, T. Suzuki, K. Iijima-Ando

Plant Genomes: From Sequence to Phenome

December 9–12 115 participants

ARRANGED BY **Gloria Coruzzi,** New York University
Michael Snyder, Yale University
Susan McCouch, Cornell University

This meeting featured 37 talks in 6 sessions, a poster session, and a workshop on Resources and New Technology. The theme was to bring plant genomics to the next level beyond sequencing, to discover gene function through phenomic approaches. A new organizational feature of the plant genome meeting was to stimulate cross-talk between plant genomics and genomics in other model organisms. To accomplish this, genome speakers from nonplant organisms were invited to speak in each session including yeast, *Caenorhabditis elegans*, and *Chlamydomonas*. Two keynote speakers opened the meeting: Claire Fraser (President of The Institute for Genomic Research) surveyed the exponential growth of sequence information from the more than 1200 genome sequencing projects under way, noting that novel, strain-specific genes were still being discovered with every new organism to be sequenced. She noted that the main limitation to linking sequence with phenotype had to do with deficiencies in annotation and in understanding the role of genes with products of unknown function. Joe Ecker presented life's complexity pyramid and outlined the effort to identify and name all the "parts" and to understand how they all work together in the context of a whole, living organism. The talks were selected from abstracts and covered progress in sessions on regulatory networks and gene expression, proteomics, phenomics, genome organization and evolution, new genomes and elements, and genomics approaches to small molecules. Speakers looked at cell-to-cell communication in the apical meristems of shoots and roots, at regulatory pathways and protein interaction networks in yeast, at clusters of genes associated with specific phenotypes in worms (phenoclusters), at MADS box genes and their relationship to petal formation, and at proteins that are required to make flagellar basal bodies in *Chlamydomonas* and their relationship to human disease. Others presented strategies for characterizing the proteome, for classifying promoters, for sequencing the maize genome, for conducting array-based genotyping, and for exploring the evolution of new cell types. There was the announcement of the new JGI effort to sequence *Selaginella*, an early, non-seed-bearing land plant that has no true leaves and represents a key node in plant evolution. Speakers provided insights into the abundance and function of small RNAs in plants, into systems for virus-induced gene silencing, and into endomembrane trafficking. The final keynote address by Marc Johnston offered a view of what is known about protein-protein interactions in *Saccharomyces cerevisiae*, with the goal of "completing yeast as an organism by 2007." The genomics workshop highlighted areas of plant genomics that are necessary for the plant community to "catch up" with the

S. McCouch, Z. Lippman

R. Martienssen, M. Snyder

genomic resources in yeast and *C. elegans*, including ORFeome, proteomics, and DNA-protein interactions. This wish list was inspired by the ideas that had been exchanged at this small but provocative conference.

PROGRAM

Keynote Addresses
C. Fraser, *The Institute for Genomic Research, Bethesda, Maryland;* J. Ecker, *The Salk Institute for Biological Studies, La Jolla, California*

Regulatory Networks and Gene Expression
Chairperson: E. Meyerowitz, *California Institute of Technology, Pasadena*

Proteomics
Chairperson: B. Chait, *The Rockefeller University, New York*

Phenomics
Chairperson: C. Boone, *University of Toronto, Canada*

Genome Organization and Evolution
Chairperson: S. Dutcher, *Washington University, St. Louis, Missouri*

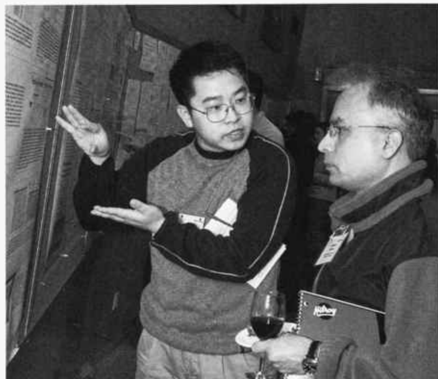
New Genomes and Elements
Chairperson: D. Weigel, *Max-Planck Institute for Developmental Biology, Tübingen, Germany*

Workshop: Resources and New Technology
Chairpersons: J. Ecker, *Salk Institute for Biological Studies, La Jolla, California;* D. Preuss, *University of Chicago, Illinois;* M. Snyder, *Yale University, New Haven, Connecticut*

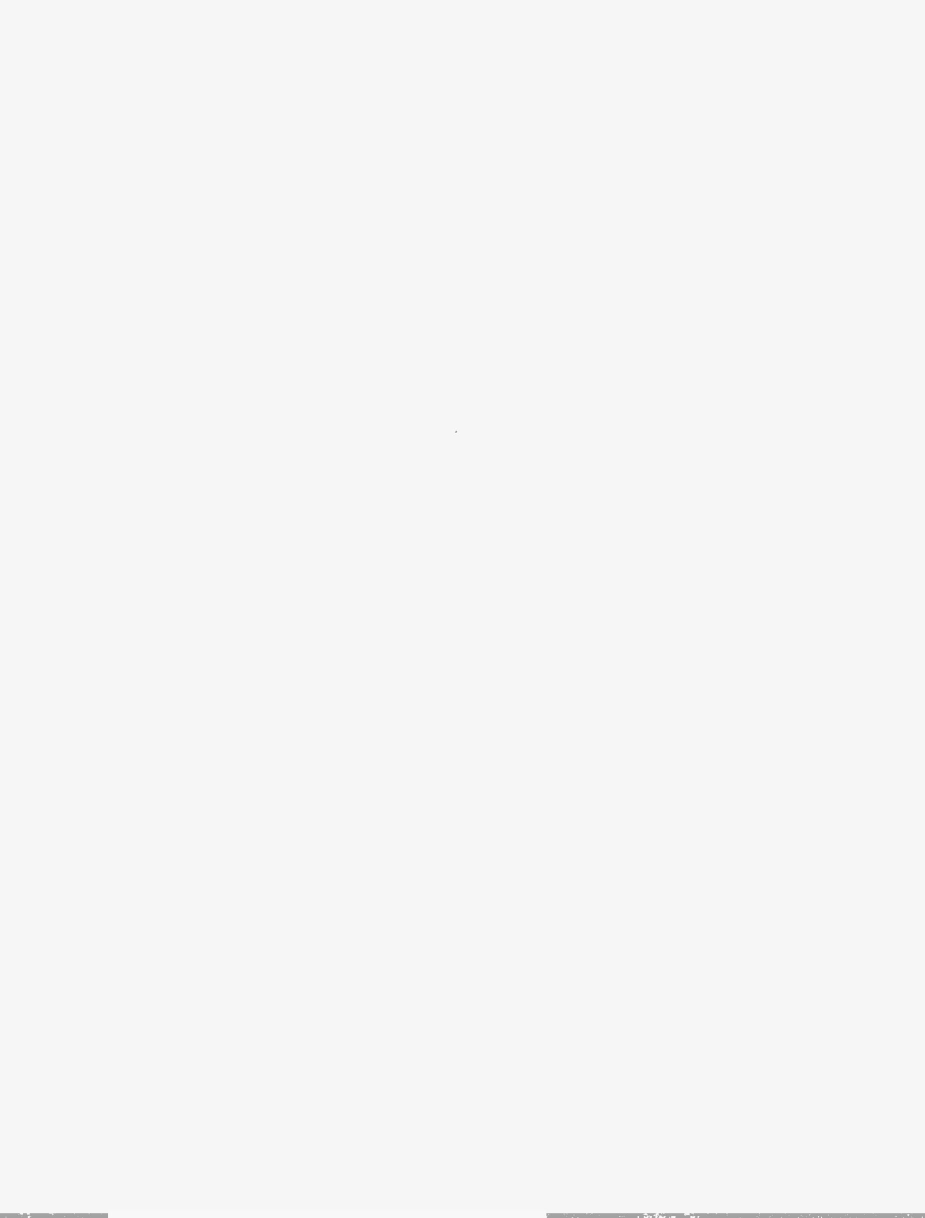
Keynote Speaker
Chairperson: M. Johnston, *Washington University School of Medicine, St. Louis, Missouri*

Genomics Approaches to Small Molecules
Chairperson: N. Raikhel, *University of California, Riverside*

G. Coruzzi, R. Flavell



Q. Dong, S. Gopalan



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 14–27

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
N. Thompson, University of Wisconsin, Madison
O. Tsai, M.D. Anderson Cancer Center/University of Texas, Houston
W.-W. Tsai, M.D. Anderson Cancer Center/University of Texas, Houston
C. Winkler, University of California, Los Angeles
N. Zenkin, Rutgers University, New Brunswick, New Jersey

This course was for scientists who were not familiar with techniques of protein isolation and characterization. It was 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 reversed-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. This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Chandra, D., Ph.D., M.D. Anderson Cancer Center/University of Texas, Smithville
Conlon, F., Ph.D., University of North Carolina, Chapel Hill
Demirel, M., Ph.D., Penn State University, University Park
Elling, A., B.S., Iowa State University, Ames
Feldman, M., B.S., Tel-Aviv University, Israel
Gibson, K., Ph.D., Massachusetts Institute of Technology, Cambridge
Govindarajan, V., Ph.D., Baylor College of Medicine, Houston, Texas
Kettunen, P., Ph.D., University of Bergen, Norway
Lipardi, C., Ph.D., National Institutes of Health/NCI, Bethesda, Maryland
Makarevitch, I., M.S., University of Minnesota, St. Paul
Marcand, S., Ph.D., CEA/Saclay, Gif-sur-Yvette, France
Norville, J., B.S., Massachusetts Institute of Technology, Cambridge
Phan, A.T., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Sformo, T., M.S., University of Alaska, Fairbanks
Tavanez, J., B.S., Hospital Santa Maria, Lisbon, Portugal
Tudor, H., Ph.D., Hospital for Special Surgery, New York

SEMINARS

Bergendahl, V., University of Wisconsin, Madison: Use of LRET-based assays for drug discovery and biochemistry; RNA polymerase–sigma factor interactions.
Burgess, R., University of Wisconsin, Madison: Introduction to protein purification and immunoaffinity chromatography.
Courey, A., University of California, Los Angeles: Sumoylation and the response to stress.
Hart, G., Johns Hopkins University, Baltimore, Maryland: Protein glycosylation: The most ubiquitous posttranslational event in eukaryotes.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Protein crystallography intro—The PAZ domain of Argonaute-2: siRNA at RISC.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Novel approach to understanding prostate cancer bone metastasis.
Mische, S., Boehringer-Ingelheim, Ridgefield, Connecticut: Protein purification and characterization for drug discovery.
Muir, T., The Rockefeller University, New York: Cutting and pasting proteins: What's happening with inteins.
Severinov, K., Rutgers University, New Brunswick, New Jersey: Role of RNA polymerase core enzyme in transcription initiation.
Stillman, B., Cold Spring Harbor Laboratory: The origin recognition complex and the chromosome inheritance cycle.

Cell and Developmental Biology of *Xenopus*

April 16–27

INSTRUCTORS **K. Cho,** University of California, Irvine
J. Christian, Oregon Health and Science University, Portland

ASSISTANTS **I. Blitz,** University of California, Irvine
G. Dalgin, Oregon Health and Science University, Portland
D. Goldman, Oregon Health and Science University, Portland
T. Nakayama, University of Virginia, Charlottesville

The frog *Xenopus* is an important vertebrate model for studies of maternal factors, regulation and molecular mechanisms of tissue inductions, and regulation of cell fate decisions. In addition, *Xenopus* oocytes and embryos provide a powerful system in which to conduct a number of cell biological and gene regulation assays. This course provided extensive laboratory exposure to the biology, manipulation, and use of oocytes and embryos of *Xenopus*. The course consisted of intensive laboratory sessions, supplemented by daily lectures and demonstrations from experts in cellular, experimental, and molecular development. Areas covered included (1) care of adults; (2) oocyte isolation and embryo production; (3) stages of embryonic development and anatomy; (4) whole-mount in situ hybridization and immunocytochemistry; (5) microinjection of eggs and oocytes with lineage tracers, DNA constructs, mRNA, and antisense oligonucleotides; (6) micromanipulation of embryos, including explant and transplantation assays; (7) in vivo time-lapse confocal imaging; (8) preparation of transgenic embryos; and (9) use of *Xenopus tropicalis* for genetic analyses. This course was suited both for investigators who

have had no experience with *Xenopus* and for those who have worked with *Xenopus* and wish to learn new and cutting-edge techniques. All applicants had current training in molecular biology and some knowledge of developmental biology. Guest lecturers included Amaya Enrique, Ken Cho, John Gurdon, Janet Heasman, Ray Keller, and Mark Mercola.

PARTICIPANTS

Boulware, M., Ph.D., University of Minnesota, Minneapolis
Burns, M., Ph.D., University of Virginia, Charlottesville
Chen, Y., Ph.D., Medical University of South Carolina, Charleston
Handrigan, G., B.S., Dalhousie University, Halifax, Nova Scotia, Canada
Heine, D., M.S., University of Aberdeen, Scotland, United Kingdom
Islam, A., M.Phil., Tottori University, Yonago, Japan
Kieckens, L., B.S., Katholieke Universiteit Leuven, Belgium
Marinescu, V., Ph.D., Children's Hospital Boston, Massachusetts
Nichane, M., M.S., Free University of Brussels, Belgium
Ny, A., Ph.D., Katholieke Universiteit Leuven, Belgium
Parker, B., M.S., Edison Biotechnology Institute/Ohio University, Athens
Rangarajan, J., M.S., National Institutes of Health, Bethesda, Maryland
Rouhana, L., Ph.D., University of Wisconsin, Madison
Vanamala, S., M.V.S., Institut de Recherches Cliniques de Montreal, Quebec, Canada
Wang, B., Ph.D., Weill Medical College of Cornell University, New York
Ware, S., Ph.D., Baylor College of Medicine, Houston, Texas

SEMINARS

Amaya, E., University of Cambridge, United Kingdom: Functional genomics in *Xenopus*.
Cho, K., University of California, Irvine: Genomic approach to studying BMP signaling.
Gurdon, J., Wellcome Trust, Cambridge, United Kingdom: Nuclear reprogramming.
Heasman, J., Children's Hospital Medical Center, Cincinnati, Ohio: Antisense technology to study maternal gene products.
Keller, R., University of Virginia, Charlottesville: The cell movements, patterning, and biomechanical gastrulation in amphibians.
Mead, P., St. Jude Children's Research Hospital, Memphis, Tennessee: Insertional mutagenesis using transposons.
Mercola, M., Burnham Institute, San Diego, California: Heart induction.
Niehrs, C., Deutsches Krebsforschungszentrum, Heidelberg, Germany: Large-scale analysis of embryonic gene expression in *Xenopus*.

Genetics of Complex Human Diseases

June 9–15

INSTRUCTORS **A. Al-Chalabi,** FRCP, Institute of Psychiatry, Kings College, London, United Kingdom
L. Almasy, Southwest Foundation for Biomedical Research, San Antonio, Texas

Complex diseases are conditions that are influenced by the actions of multiple genes, their interactions with each other and with the environment. This lecture course considered the difficulties in studying the genetic basis of complex disorders such as diabetes, cardiovascular disease, cancer, Alzheimer's disease, schizophrenia, and epilepsy. We discussed genetic-epidemiologic study designs, including family, twin, case/control, and adoption studies, as well as methods for quantifying the strength of the genetic influences on a disease. A major focus was the identification of specific gene effects using both linkage and association analyses and their variants. We discussed the efficiency and robustness of different designs for such analysis and how evidence from epidemiologic studies informs both the design and interpretation of molecular genetic studies. Study design and methods for analysis of quantitative risk factors related to complex diseases were covered, as well as the latest ideas in data reduction such as haplotype mapping and SNP tagging. An overview of high-throughput laboratory methods was given to provide participants with insight into the applications of these techniques. Illustrations were provided through discussion of results from ongoing studies of a variety complex diseases and related risk factors.

PARTICIPANTS

Barreto, M., B.S., Instituto Gulbenkian de Ciencia, Oeiras, Portugal
Body, S., M.D., Brigham & Women's Hospital, Boston, Massachusetts
Buervenich, S., Ph.D., National Institutes of Mental Health, Bethesda, Maryland
Clarimon, J., Ph.D., National Institutes of Health, Rockville, Maryland
Congdon, E., M.A., Stony Brook University, New York
Davuluri, R., Ph.D., Ohio State University, Columbus
De Jager, P., Ph.D., Harvard Medical School, Boston, Massachusetts
Duga, S., B.S., University of Milan, Italy
Ferguson, B., Ph.D., Oregon National Primate Research Center, Beaverton
He, Q., B.S., Michigan State University, East Lansing
Morley, M., M.S., Children's Hospital of Philadelphia, Pennsylvania
Rasmussen, E., Ph.D., Roche Pharmaceuticals, Nutley, New Jersey
Rosen, M., M.D., University of California, San Francisco
Schlesinger, D., M.D., University of Sao Paulo, Brazil
Simen, A., Ph.D., Yale University, New Haven, Connecticut
Stambolian, D., Ph.D., University of Pennsylvania, Philadelphia
Traynor, B., M.D., Massachusetts General Hospital, Charlestown
Tukel, T., Ph.D., Mt. Sinai School of Medicine, New York
Viel, K., B.S., Emory University, Atlanta, Georgia
Wanke, K., Ph.D., National Cancer Institute, Bethesda, Maryland

SEMINARS

Al-Chalabi, A., Institute of Psychiatry, Kings College, London, United Kingdom: An introduction to genetic linkage.
Almasy, L., Southwest Foundation for Biomedical Research, San Antonio, Texas: An introduction to variance components.
Altshuler, D., Massachusetts General Hospital, Harvard Medical School, Boston: The International Hap Map Project.
Blangero, J., Southwest Foundation for Biomedical Research, San Antonio, Texas: From QTL to gene.
Borecki, I., Washington University School of Medicine, St. Louis, Missouri: Study designs.
Edenberg, H., Indiana University, Indianapolis: High-throughput laboratory methods.
Goldstein, D., University College London, United Kingdom: Data reduction, SNP tagging, and pharmacogenomics.
Knight, J., Institute of Psychiatry, London, United Kingdom: Association studies; TDT-based methods.
Krainer, A., Cold Spring Harbor Laboratory: Genetic mechanisms in complex diseases.
Merikangas, K., National Institute of Mental Health, Bethesda, Maryland: Genetic epidemiological approaches to complex disorders.
Ottman, R., Columbia University School of Public Health, New York: An introduction to complex genetics.

Advanced Bacterial Genetics

June 9–29

INSTRUCTORS
K. Hughes, University of Washington, Seattle
U. Jenal, University of Basel, Switzerland
K. Pogliano, University of California, San Diego, La Jolla

ASSISTANTS
D. Broder, University of California, San Diego, La Jolla
B. Christen, University of Basel, Switzerland
C. Wozniak, University of Washington, Seattle

The course presented logic and methods used in the genetic dissection of complex biological processes in eubacteria. Laboratory methods 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 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. This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Black, S., B.S., University of Aberdeen, United Kingdom
Bjornsdottir, S., B.S., University of Iceland, Reykjavik
Chevance, F., Ph.D., University of Konstanz, Germany
Colangeli, R., Ph.D., University of Medical & Dentistry of New Jersey, Newark
Drummond, D.A., B.S.E., California Institute of Technology, Pasadena
Frank, K., B.S., Mayo Clinic, Rochester, Minnesota
Gescher, J., Diplom., University of Freiburg, Germany
Huang, K. C., B.S., Massachusetts Institute of Technology, Cambridge
Klausen, M., M.S., Technical University of Denmark
Konjufca, V., Ph.D., Washington University, St. Louis, Missouri
Raffatellu, M., M.D., Texas A&M University, College Station
Schneeweiss, J., B.S., University of Oxford, United Kingdom
Shanks, R., Ph.D., Dartmouth Medical School, Hanover, New Hampshire
Thompson, K., B.S., National Cancer Institute, Bethesda, Maryland
Turner, S., Ph.D., University of Auckland, New Zealand
Wolf, D., Ph.D., Lawrence Berkeley National Labs., Berkeley, California

SEMINARS

Adhya, S., National Institutes of Health/NCI, Bethesda, Maryland: Structure, assembly, and dynamics of a gene regulatory nucleoprotein complex containing a DNA loop.
Brun, Y., Indiana University, Bloomington: Generation and maintenance of cellular asymmetry in *Caulobacter*.
Errington, J., University of Oxford, United Kingdom: How *Bacillus subtilis* finds the mid-cell site for division and avoids bisecting its chromosome.
Gross, C., University of San Francisco, California: Sensing and responding to envelope stress.
Harshey, R., University of Texas, Austin: Surface sensing: Swarming motility in *S. typhimurium*.
Maloy, S., San Diego State University, California: Genetic basis of host specificity in *Salmonella*.
Nystrom, T., Goteborg University, Sweden: RpoS regulation in stationary phase.
Otteman, K., University of California, Santa Cruz: Swimming in the stomach: How chemotaxis helps *Helicobacter pylori* infect.
Taylor, R., Dartmouth Medical School, Hanover, New Hampshire: The role of pili in *Vibrio cholerae* infection.
Weinstock, G., Baylor College of Medicine, Houston, Texas: From genetics to genomics.

Ion Channel Physiology

June 9–29

INSTRUCTORS
M. Farrant, University College London, United Kingdom
M. Hausser, University College London, United Kingdom
N. Spruston, Northwestern University, Evanston, Illinois

ASSISTANTS
B. Clark, University College London, United Kingdom
T. Jarsky, Northwestern University, Chicago, Illinois
B. Stell, University of California, Los Angeles

The primary goal of this course was to investigate, through lectures and laboratory work, the properties of ion channels that allow neurons to carry out their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitter at central and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, (3) respond to neuromodulators with changes in functional properties, or (4) are developmentally required and regulated. The research interests of guest lecturers reflected these areas of emphasis.

The laboratory component of the course introduced students to electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice preparations. Different recording configurations were used (e.g., whole-cell, cell-free, and nucleated patches)

to examine macroscopic or single-channel activity. Similarly, various methods of ligand and drug application were demonstrated. The advantages and disadvantages of each method, preparation, and recording technique were considered in relation to the specific scientific questions being asked.

PARTICIPANTS

Barker, L., B.A., University of California, San Francisco
Buonanno, A., Ph.D., National Institutes of Health, Bethesda, Maryland
Cooke, B., Ph.D., Northwestern University, Evanston, Illinois
Fisher, T., B.S., Wake Forest University, Winston-Salem, North Carolina
Jocoy, E., B.S., University of California, Los Angeles
Luu, P., Ph.D., University of California, Berkeley
Nair, A., Ph.D., University of Cambridge, United Kingdom
Norris, A., B.A., Washington University, St. Louis, Missouri
Nuriya, M., B.S., Johns Hopkins University, Baltimore, Maryland
Petreanu, L., Ph.D., Cold Spring Harbor Laboratory
Sandberg, M., Ph.D., Karolinska Institutet, Stockholm, Sweden
Sarah, P., B.S., University of Bern, Switzerland

SEMINARS

Bean, B., Harvard Medical School, Boston, Massachusetts: Channel integration and firing.
Diamond, J., National Institutes of Health, Bethesda, Maryland: Glutamate EPSCs and uptake.
Fitzsimonds, R., Yale University School of Medicine, New Haven, Connecticut: Synaptic plasticity during development.
Jonas, P., Albert-Ludwigs-Universitat, Freiburg, Germany: Cortical microcircuits.
Larkum, M., University of Bern, Switzerland: Dendritic integration.
Llinas, R., New York University School of Medicine, New York: Ion channels and integration.
Nerbonne, J., Washington University School of Medicine, St. Louis, Missouri: Voltage-gated K channels in heart and neurons.
Nicoll, R., University of California, San Francisco: Cannabinoids and synaptic plasticity.
Nusser, Z., Institute of Experimental Medicine, Budapest: Hyperpolarization-activated cation channels.
Raman, I., Northwestern University, Evanston, Illinois: Action potential, Hodgkin-Huxley.
Raman, I., Northwestern University, Evanston, Illinois: Voltage-gated Na channels.
Scanziani, M., University of California, San Diego, La Jolla: Synaptic plasticity.
Soltesz, I., University of California, Irvine: GABA receptors and inhibition.
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 channels.

Molecular Embryology of the Mouse

June 9–29

INSTRUCTORS **T. Lufkin,** Mt. Sinai School of Medicine, New York
C. Stewart, National Cancer Institute, Frederick, Maryland

ASSISTANTS **D. Escalante-Alcalde,** Institute of Cellular Physiology-UNAM, Mexico
J. Liu, Mt. Sinai School of Medicine, New York

This intense laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse embryonic development. Laboratory components provided an introduction into the technical aspects of working with and analyzing mouse embryos, and lectures provided the conceptual basis for contemporary research in mouse development. Experimental techniques covered in the practicals included in vitro culture and manipulation of pre- and postimplantation embryos; transgenesis by DNA microinjection, embryo transfer, establishment, culture, and genetic manipulation of embryonic stem cells; production of chimeras by aggregation with and injection of embryonic stem cells; and the analysis of development by whole-mount in situ hybridization, skeletal preparation, and transgene expression.

PARTICIPANTS

Attie, A., Ph.D., University of Wisconsin, Madison
Bennett, V., Ph.D., West Virginia University School of Medicine, Morgantown
Chal, J., M.S., Stowers Institute for Medical Research, Kansas City, Missouri
Herrera, E., Ph.D., Columbia University, New York
Jackerott, M., Ph.D., University of Copenhagen, Denmark
Katusic A., B.S., University of Zagreb, Croatia
Kobayashi, Y., Ph.D., University of Iowa College of Medicine, Iowa City
Mayer, A., Ph.D., Medical College of Wisconsin, Milwaukee
Murchison, E., B.S., Cold Spring Harbor Laboratory
Musacchio, M., Ph.D., University of California, Irvine
Ostman, N., M.S., Turku Center of Biotechnology, Finland
Stauber, M., Ph.D., Cancer Research UK, London, United Kingdom
Wamstad, J., B.A., University of Minnesota
Wuelling, M., Ph.D., Max-Planck Institute for Molecular Genetics, Berlin, Germany

SEMINARS

Behringer, R., M.D. Anderson Cancer Center/University of Texas, Houston: Gene targeting 101 and conditional genetic manipulation.
Bestor, T., Columbia College of Physicians & Surgeons, New York: Epigenetics and DNA methylation.
Brannstrom, M., The Sahlgrenska Academy, Goteborg, Sweden: Surgical transplantation of uteri.
Capel, B., Duke University Medical Center, Durham, North Carolina: Gonad organogenesis.
Duncan, S., Medical College of Wisconsin Neurobiology and Anatomy, Madison: Genetic regulation of organogenesis: Liver development.
Heard, E., Curie Institute, Paris, France: Dynamics of X-chromosome inactivation.
Jenkins, N., National Cancer Institute, Frederick, Maryland: Mouse genetics.
Jessell, T., Columbia University, New York: Neural development.
Joyner, A., New York University Medical Center, New York: Genetic approaches in mouse to study CNS patterning.
Ko, M., National Institute of Aging, Baltimore, Maryland: Gene arrays and the analysis of mouse development.
Lewandoski, M., CDBL, National Cancer Institute, Frederick, Maryland: Genetic approaches to studying signaling in limb development.
Lovell-Badge, R., National Institute for Medical Research, London, United Kingdom: Mammalian sex determination.
Lufkin, T., Mount Sinai School of Medicine, New York: Homeobox gene control of embryonic patterning.
Magnuson, T., University of North Carolina, Chapel Hill: Genetic approaches to development.
McLaren, A., Wellcome/CRC Institute, Cambridge, Massachusetts: Germ cells.
Nagy, A., Mount Sinai Hospital/Samuel Lunenfeld Research Institute, Ontario, Canada: ES cells and chimeras.
Overbeek, P., Baylor College of Medicine, Houston, Texas: Transposon-mediated mutagenesis.
O'Brien, T., Jackson Laboratory, Bar Harbor, Maine: An enhanced GFP-based dual-color reporter to recover mutations in mice.
Perry, T., RIKEN Center for Developmental Biology, Kobe, Japan: Micromanipulation of embryos and for ICSI, transgenesis, and nuclear transplantation.
Rosenthal, N., EMBL-Monterotondo, Rome, Italy: Heart development.
Rossant, J., Samuel Lunenfeld Research Institute, Ontario, Canada: Regulation of extraembryonic development.
Rowitch, D., Dana-Farber Cancer Institute, Harvard University, Boston, Massachusetts: *Gal4/Uas* binary gene system and conditional gene expression.
Sharpe, J., MRC Human Genetics Unit, Edinburgh, Scotland: OPT microscopy and the Edinburgh mouse atlas.
Shen, M., UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey: Left-right patterning.
Solter, D., Max-Planck Institute, Freiburg, Germany: From egg to blastocyst: Hard road to follow.
Stewart, C., National Cancer Institute, Frederick, Maryland: Early mouse development and implantation.
Stewart, A.F., Dresden University of Technology, Germany: Targeting strategies: Their design, construction, and integration.
Tam, P., Children's Medical Research Institute, Wentworthville, Australia: Overview of mouse development and genetics. Gastrulation and morphogenesis: Building the body plan.
Threadgill, D., University of North Carolina, Raleigh-Durham: Quantitative traits and bioinformatics.
Yamaguchi, T., CDBL, National Cancer Institute, Frederick, Maryland: Wnt pathways in development.

Computational Neuroscience: Vision

June 18–July 1

INSTRUCTORS **J. Demb,** University of Michigan, Ann Arbor
P. Glimcher, New York University, New York
E. Simoncelli, New York University, New York

ASSISTANTS **B. Borghuis,** Utrecht University, The Netherlands
N. Rust, New York University, New York

Computational approaches to neuroscience produced important advances in our understanding of neural processing. Prominent success came in areas where strong inputs from neurobiological, behavioral, and computational investigation can interact. The theme of the course was that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions helped guide research in neuroscience. Through a combination of lectures and hands-on experience in a computer laboratory, this intensive course examined color vision, spatial pattern analysis, motion analysis, oculomotor function, attention, and decision-making. Lecturers this year included Edward Adelson, David Brainard, Matteo Carandini, Eduardo Chichilnisky, Kathleen Cullen, Bruce Cumming, James DiCarlo, David Heeger, Daniel Kersten, J. Anthony Movshon, Pamela Reinagel, Fred Rieke, Ruth Rosenholtz, Michael Shadlen, Stefan Treue, and Preeti Verghese. The

course was held at the Laboratory's Banbury Conference Center located on the north shore of Long Island. All participants stay within walking distance of the Center, close to the tennis court, pool, and private beach.

PARTICIPANTS

Amstrong, K., B.S.E., Stanford University, California
Basole, A., B.S., Duke University Medical Center, Durham, North Carolina
Bonin, V., M.S., The Smith-Kettlewell Eye Research Institute, San Francisco, California
Challinor, K., B.Psych., Macquarie University, Sydney, Australia
Cohen, M., B.S., Stanford University, California
Doerschner, K., B.A., New York University, New York
Dunn, F., B.S., University of Washington, Seattle
Field, G., B.S., University of Washington, Seattle
Hanks, T., B.S., University of Washington, Seattle
Larsson, J., Ph.D., New York University, New York
Liston, D., B.S., Salk Institute, La Jolla, California
Maciokas, J., Ph.D., University of California, Davis
Macuga, K., B.S., University of Califonria, Santa Barbara
Malone, B., Ph.D., University of California, Los Angeles
Mitchell, J., Ph.D., The Salk Institute, La Jolla, California
Naito, T., Ph.D., Osaka University, Japan
Nienborg, H., M.D., National Eye Institute/NIH, Bethesda, Maryland
Pieper, F., Diplom., German Primate Center, Gottingen, Germany
Sather, B., Ph.D., University of Pittsburgh, Pennsylvania
Schoppik, D., B.A., University of California, San Francisco
Sundberg, K., B.S., The Salk Institute, La Jolla, California
Tailby, C., Ph.D., New York University, New York
Yin, L., B.S., University of Pennsylvania, Philadelphia
Zemach, I., M.S., University of Washington, Seattle

SEMINARS

Brainard, D., University of Pennsylvania, Philadelphia; Adelson, E., Massachusetts Institute of Technology, Cambridge: Constancy/adaptation; Context; Trichromacy; Elements of early vision.
Carandini, M., The Smith-Kettlewell Eye Research Institute, San Francisco, California; Simoncelli, E., New York University, New York: V1 physiology II; Image statistics II: Sparseness/ICA/nonlinearities.
Chichilnisky, E.J., The Salk Institute, La Jolla, California: Linear systems; Linearity in the retina.
Chichilnisky, E.J., The Salk Institute, La Jolla, California; Demb, J., University of Michigan, Ann Arbor: Retinal gaglion cell encoding; RGC nonlinearities.
Chichilnisky, E.J., The Salk Institute, La Jolla, California; Brainard, D., University of Pennsylvania, Philadelphia: RGC population code; Linear algebra.
Cullen, K., McGill University, Montreal, Canada: Eye movement.
Cumming, B., National Eye Institute/NIH, Bethesda, Maryland: Binocular stereo: Perception and physiology.
Demb, J., University of Michigan, Ann Arbor; Glimcher, P., New York University, New York; Simoncelli, E., New York University, New York: Course themes: Tuning/population/adaptation/estimation.
Demb, J., University of Michigan, Ann Arbor; Chichilnisky, E.J., The Salk Institute, La Jolla, California: RGC nonlinear mechanisms; RGC contrast adaptation.
DiCarlo, J., HHMI/Baylor College of Medicine, Houston, Texas: Object/shape physiology.
Glimcher, P., New York University, New York: Decision/games.
Heeger, D., Stanford University, California: Human V1/fMRI.
Kersten, D., Max-Planck Institute, Tübingen, Germany: Object/ shape perception.
Movshon, J.A., New York University, New York: MT physiology.
Reinagel, P., University of California, San Diego, La Jolla; Simoncelli, E., New York University, New York: LGN; Image Statistics I. PCA/SpectralModels.
Reinagel, P., University of California, San Diego, La Jolla; Carandini, M., The Smith-Kettlewell Eye Research Institute, San Francisco, California: Efficient coding; V1 Physiology I.
Rieke, F., University of Washington, Seattle: Signal detection theory; Rods/photos; Entropy/information; Spikes.
Rosenholtz, R., NASA Ames, Moffett Field, California: Pattern perception/texture.
Shadlen, M., University of Washington, Seattle: Motion perception/decisions.
Treue, S., German Primate Center, Goettingen, Germany: Attention physiology.
Verghese, P., The Smith-Kettlewell Eye Research Institute, San Francisco, California: Attention psychophysics.

DNA Microarray Applications

June 22–29

INSTRUCTORS

V. Iyer, University of Texas, Austin
A. Khodursky, University of Minnesota, St. Paul
J. Lieb, University of North Carolina, Chapel Hill

ASSISTANTS

M. Buck, University of North Carolina, Chapel Hill
P. Killion, University of Texas, Austin
J. Kim, University of Texas, Austin
P. Mieczkowski, University of North Carolina, Chapel Hill

DNA microarrays are simple, inexpensive, and versatile tools for experimental explorations of genome structure, gene expression programs, gene function, and cell and organismal biology. In this hands-on eight-day course, students were introduced to the underlying technology, and then designed and carried out a number of experiments for analysis by DNA microarray hybridization, data analysis, display, and interpretation. Experimental applications covered in the course included systematic studies of global gene expression programs, inferring gene function using microarrays, genome-wide DNA-protein interactions, and measuring changes in gene copy number. Guest lecturers presented state-of-the-art new technology, experimental applications, and interpretation of large genomic data sets. Students who completed this course were equipped with the skills and know-how to introduce or apply DNA microarray technology to their own laboratories or institutions. This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Aguilera Alvarez, C., M.S., Catholic University of Chile, Santiago
Bak, M., M.S., University of Copenhagen, Denmark
Butchko, R., B.S., United States Department of Agriculture, Peoria, Illinois
Chen, H., M.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Enmark, E., B.S., Karolinska Institutet, Huddinge, Sweden
Hubal, M., B.S., University of Massachusetts, Amherst
Meza-Zepeda, L., Ph.D., The Norwegian Radium Hospital, Oslo, Norway
Miller, K., Ph.D., Fisheries and Oceans, British Columbia, Canada
Nally, J., Ph.D., University of California, Los Angeles
Nielsen, K., Ph.D., Danish Institute for Food and Veterinary Research, Copenhagen, Denmark
Orlando, E., Ph.D., St. Mary's College of Maryland, St. Mary's City
Rada, A., B.S., Uppsala University, Sweden
Sloane, M., B.S., Vienna University, Austria
Sobko, A., Ph.D., IOGEN Corporation, Ontario, Canada
Straub, P., Ph.D., Richard Stockton College, Pomona, New Jersey
Suarez-Castillo, E., B.S., University of Puerto Rico, San Juan
Subramaniam, S., M.S., Centre for Cellular & Molecular Biology, Hyberabad, India
Tammoja, K., M.S., National Institutes for Health Development, Tallinn, Estonia
Wang, C. K., Ph.D., Medimmune Vaccines, Mountain View, California
Yu, M., Ph.D., Cold Spring Harbor Laboratory

SEMINARS

Botstein, D., Princeton University, New Jersey: Functional genomics: Getting beyond descriptive studies.
Buck, M., University of North Carolina, Chapel Hill: Analysis of ChIP-ChiP data.
Churchill, G., The Jackson Laboratory, Bar Harbor, Maine: Statistical design and analysis of gene expression microarray data.
Diaz, E., University of California, Berkeley: Using R for microarray data analysis.
Khodursky, A., University of Minnesota, St. Paul: Advanced data analysis and statistical considerations.
Killion, P., University of Texas, Austin: Submitting and retrieving data with the longhorn array database; Advanced features of the longhorn array database.
Mieczkowski, P., University of North Carolina, Chapel Hill: Data analysis for aCGH experiments.
Murphy, C., University of California, San Francisco: Experimental design from a biologist's point of view.
Sherlock, G., Stanford University, California: Analysis and organization of DNA microarray data.
Sherlock, G., Stanford University, California: Analysis and organization of DNA microarray data II.
Verdnik, D., Axon Instruments, Foster City, California: Microarray scanning and quantitation with Genepix Pro; Submitting data to acuity; Advanced features of acuity.
Whitfield, M., Dartmouth Medical School, Stanford, California: Considerations for the design and analysis of time-course experiments.

Advanced Techniques in Plant Science

July 1–21

INSTRUCTORS	**J. Bender,** Johns Hopkins University School of Public Health, Baltimore, Maryland **L. Hobbie,** Adelphi University, Garden City, New York **H. Ma,** Penn State University, University Park **S. McCormick,** Plant Gene Expression Center, Albany, California **R. Pruitt,** Purdue University, W. Lafayette, Indiana
ASSISTANTS	**S. Knapke,** Purdue University, W. Lafayette, Indiana **W. Li,** Penn State University, University Park **D. Love,** Adelphi University, Garden City, New York **P. Woo,** Adelphi University, Garden City, New York

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 (development of flowers, leaves, male and female gametophytes, and embryos); perception of light and photomorphogenesis; 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 sessions provided an introduction to important techniques currently used in plant research. These included studies of plant development, meiosis, pollen development, fern reproduction, plant cell-imaging techniques, laser-assisted tissue microdissection, mutant analysis, transient gene expression, applications of green fluorescent protein fusions, proteomics approaches, and techniques commonly used in genetic and physical mapping. The course also included several short workshops on important themes in genetics and a short workshop on scientific writing. This course was supported by the National Science Foundation.

PARTICIPANTS

Bernacki, S., North Carolina State University, Raleigh
Bliss, B., M.S., Penn State University, University Park
Brown, L.-A., B.S., University of Leeds, United Kingdom
Costa, S., Lic., Guilbenkian Institute for Science, Oeiras, Portugal
Johansen, W., M.S., Hedmark University College, Hamar, Norway
Kaufmann, K., Diplom., FSU Jena, Germany
Klanrit, P., B.S., Purdue University, W. Lafayette, Indiana
Levall, M., B.S., Syngenta Seeds AB, Landskrona, Sweden
Levesque, E., B.A., The Arnold Arboretum of Harvard University, Cambridge, Massachusetts
Liang, H., Ph.D., Penn State University, University Park
Romero, C., M.S., International Center for Tropical Agriculture, Cali, Colombia
Ron, M., M.S., Tel-Aviv University, Israel
Svennerstam, H., M.S., Swedish University of Agricultural Sciences, Umea, Sweden
Thingnaes, E., Ph.D., Agricultural University of Norway, Aas
Vandesteene, L., M.S., Catholic University Leuven, Belgium
Ye, S., M.A., University of Minnesota, St. Paul

SEMINARS

Banks, J., Purdue University, W. Lafayette, Indiana: Gametophytes and evolution.
Bender, J., Johns Hopkins University School of Public Health, Baltimore, Maryland: Epigenetics I.
Bowman, J., University of California, Davis: Leaves; Mutagenesis.
Briggs, W., Carnegie Institution of Washington, Stanford, California: Light signaling.
Grossniklaus, U., University of Zurich, Switzerland: Female gametophyte.
Hangarter, R., Indiana University, Bloomington: Light responses.
Hobbie, L., Adelphi University, Garden City, New York: Auxin signaling; Genetics workshop I; Genetics workshop II.
Jackson, D., Cold Spring Harbor Laboratory: Plant cell-cell communication.
Kao, T., Penn State University, University Park: Self-incompatibility.
Kellogg, E., University of Missouri, St. Louis: Evolution of regulatory genes; Phylogenetics.
Liu, Z., University of Maryland, College Park: Flower development.
Lukowitz, W., Cold Spring Harbor Laboratory: Embryo development.
Ma, H., Penn State University, University Park: Plant anatomy; Microsporogenesis.
Malamy, J., University of Chicago, Illinois: Roots.
May, B., Cold Spring Harbor Laboratory: Enhancer trap.
McCormick, S., Plant Gene Expression Center, Albany, California: Pollen.
McCourt, P., University of Toronto, Ontario, Canada: Hormone networks.
McNellis, T., Penn State University, University Park: Plant-pathogen interactions.
Nelson, T., Yale University, New Haven, Connecticut: Vascular development.
Nuhse, T., The Sainsbury Laboratory, Norwich, United Kingdom: Proteomics.
Pruitt, R., Purdue University, W. Lafayette, Indiana: Organ fusion.
Reiser, L., Carnegie Institution of Washington, Stanford, California: Database mining.
Richards, E., Washington University, St. Louis, Missouri: Epigenetics II.
Schaller, E., Dartmouth College, Hanover, New Hampshire: Ethylene.
Sussex, I., Yale University, New Haven, Connecticut: Introduction to plants.
Timmermans, M., Cold Spring Harbor Laboratory: Maize shoot patterning.
Vaugh, M., Cold Spring Harbor Laboratory: Enhancer trap.

Neurobiology of *Drosophila*

July 2–22

INSTRUCTORS	**R. Baines,** University of Warwick, Coventry, United Kingdom **S. De Belle,** University of Nevada, Las Vegas **D. Van Vactor,** Harvard Medical School, Boston, Massachusetts
COINSTRUCTORS	**K. Johnson,** Harvard Medical School, Boston, Massachusetts **J. Waters Shuler,** Harvard Medical School, Boston, Massachusetts
ASSISTANTS	**K. Miller,** Harvard Medical School, Cambridge, Massachusetts **E. Pym,** University of Warwick, Coventry, United Kingdom **C. Serway,** University of Nevada, Las Vegas

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students through established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The three-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that 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 immunohistochemistry, labeling of identified neurons, electrophysiological recording from nerves and muscles, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system. This course was supported with funds provided by the National Institute of Mental Health and the Howard Hughes Medical Institute.

PARTICIPANTS

Butler, C., Ph.D., University of Pennsylvania School of Medicine, Philadelphia
Cziko, A-M., B.S., University of Arizona, Tucson
DePace, A., Ph.D., University of California Berkeley Center for Integrated Genomics, Berkeley
Edwards, T., B.S., Dalhousie University, Nova Scotia, Canada
Fiddian, B., Ph.D., University of Cambridge, United Kingdom
Hoyer, S., M.S., University of Wuerzburg, Germany
Li, X., Ph.D., Harvard Medical School, Boston, Massachusetts
Liu, X., M.S., Baylor College of Medicine, Houston, Texas
Long, A., B.S., University of Kentucky, Lexington
Lu, C., B.S., Brandeis University, Waltham, Massachusetts
Reddy, S., M.S., University of Pennsylvania, Philadelphia
Tasic, B., B.S., Stanford University, California

SEMINARS

Auld, V., University of British Columbia, Vancouver: Glial development; Bioinformatics 101.
Baines, R., University of Warwick, United Kingdom: Synaptic plasticity; CNS electrophysiology.
Bashaw, G., University of Pennsylvania, Philadelphia: Axon guidance.
Blau, J., New York University, New York: Rythmicity.
Cagan, R., Washington University, St. Louis, Missouri: Death and the eye.
de Belle, S., University of Nevada, Las Vegas: Behavior 101; Learning and memory: Hardware.
Dubnau, J., Cold Spring Harbor Laboratory: Learning and memory: Software.
Eberl, D., University of Iowa, Iowa City: Hearing and smell.
Godenschwege, T., University of Massachusetts, Amherst; Allen, M., University of Kent, Canterbury, United Kingdom: Giant fiber system.
Johnson, K., Pomona College, California: Neurogenesis.
Kravitz, E., Harvard Medical School, Boston, Massachusetts: Aggression.
Landgraf, M., Cambridge University, United Kingdom: Neuornal identity.
Macleod, G., University of Toronto, Canada: Imaging neural activity.
O'Dell, K., University of Glasgow, United Kingdom: Courtship.
Shaw, P., Washington Medical School, St. Louis, Missouri: Sleep.
Stewart, B., University of Toronto, Canada: Electrophysiology 101; Synaptic release.
Strauss, R., Theodor Boveri Institute, Wurzburg, Germany: Motor behavior; Visual system.
Tepass, U., University of Toronto, Canada: The fly embryo and adult.
Van Vactor, D., Harvard Medical School, Boston, Massachusetts: Genetics 101 (techniques).
Waters-Shuler, J., Harvard Medical School, Boston, Massachusetts: Light microscopy; Confocal microscopy.
Zhang, B., University of Texas, Austin: The fly NMJ.

Molecular Mechanisms of Human Neurological Diseases

July 6–12

INSTRUCTORS **S. Gandy,** Thomas Jefferson University, Philadelphia, Pennsylvania
J. Hardy, National Institutes of Health/NIA, Bethesda, Maryland
H. Orr, University of Minnesota, Minneapolis

How and why do neurons dysfunction or die in specific acute or chronic human neurological disorders? What are the molecular and biochemical manifestations of specific genetic lesions in specific neurological disorders? Do different pathological dysfunctions share common mechanisms? What practical treatments can be contemplated? This lecture course explored possible answers to these important questions. Recent advances in neurogenetics and in molecular and cell biology shed light on the mechanisms that underlie nervous system injury in disease states such as stroke, epilepsy, Alzheimer's disease, Parkinson's disease, frontotemporal dementia, prion diseases, lissencephaly, Fragile X mental retardation, paraneoplastic neurological degeneration, and polyglutamine repeat disorders. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their areas of expertise. Overview was provided, and course participants were not required to have familiarity with neurological diseases, although a background in basic nervous system structure and organization was extremely helpful.

The course focused principally on the specific hypotheses and approaches driving current research. Emphasis was placed on the highly dynamic interface between basic and clinical investigation, including the interdependence of clinical research and disease model development, and the value of disease research in understanding the normal function of the nondiseased nervous system. The course was held at the Laboratory's Banbury Conference Center located on the north shore of Long Island. All participants stay within walking distance of the Center, close to tennis court, pool, and private beach. In 2004, partial support for the course was provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Bandopadhyay, R., Ph.D., Royal Free and University College London Medical School, United Kingdom
Berger, Z., B.S., University of Cambridge, United Kingdom
Betts, J., B.S., University of Newcastle Upon Tyne, United Kingdom
Englund, U., Ph.D., H. Lundbeck A/S, Copenhagen, Denmark
Greenway, M., M.D., National Centre for Medical Genetics, Dublin, Ireland
Gregory, G., B.T., Prince of Wales Medical Research Institute, Sydney, Australia
Hasegawa, S., M.S., University of Tokyo, Japan
Hong, S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Katzov, H., B.S., Centre for Genomics and Bioinformatics, Stockholm, Sweden
Leblanc, G., Ph.D., National Institutes of Health/NINDS, Bethesda, Maryland
Lewis, P., B.S., Institute of Neurology, London, United Kingdom
Liu, J., Ph.D., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
Munoz-Cabello, A., B.S., University of Seville, Spain
Park, L., M.S., Michigan State University, East Lansing
Ravits, J., M.D., Benaroya Research Institute, Seattle, Washington
Sanders, H., B.S., E. Carolina University Brody School of Medicine, Greenville, North Carolina
Shimoji, M., Ph.D., National Institutes of Health/NIA, Bethesda, Maryland
Zhang, L., Ph.D., Johns Hopkins University, Baltimore, Maryland

SEMINARS

Collinge, J., Imperial College/St. Mary's Hospital, London, United Kingdom: Prion diseases.
Cookson, M., National Institutes of Health/NIA, Bethesda, Maryland: Molecular pathogenesis of Parkinson's disease.
Darnell, R., HHMI/The Rockefeller University, New York: Neurobiology of the paraneoplastic neurological diseases and Fragile-X mental retardation.
Davidson, B., University of Iowa College of Medicine, Iowa City: Animal models of triplet repeat diseases.
Duff, K., New York University, Orangeburg: Tauopathies II: Animal models.
Frackowiak, R., Institute of Neurology, London, United Kingdom: Structural and functional basis of cerebral cotical function.
Gandy, S., Thomas Jefferson University, Philadelphia, Pennsylvania: Amyloid and Alzheimer's I: Molecular basis for anti-amyloid therapeutics.
Hardy, J., National Institutes of Health/NIA, Bethesda, Maryland; Gwinn Hardy, K., National Institutes of Health/NINDS, Bethesda, Maryland: Introduction to neurogenetics I; Introduction to neurogenetics II.
Holtzman, D., Washington University School of Medicine, St. Louis, Missouri: Amyloid and Alzheimer's II: Role of Abeta binding molecules and anti-Abeta antibodies in AD pathogenesis, diagnosis, and therapy.
Hutton, M., Mayo Clinic, Jacksonville, Florida: Tauopathies I: Genetics.
Iadecola, C., Weill Cornell Medical Center, New York: Molecular pathogenesis of stroke.
Macdonald, M., Massachusetts General Hospital, Charlestown: Trinucleotide repeat diseases: I–Huntington's disease.
Malinow, R., Cold Spring Harbor Laboratory: Amyloid and Alzheimer's III: Physiology of A.
McNamara, J., Duke University Medical Center, Durham, North Carolina: Cellular and molecular mechanisms of epilepsy.
Murphy, D., National Institutes of Health/NINDS, Rockville, Maryland: Extramural programs at NINDS.
Orr, H., University of Minnesota, Minneapolis: II: Spinocerebellar ataxia.
Siegel, J., Veterans Affairs Greater Los Angeles Health Care System, North Hills, California: Hypocretin (orexin): Role in narcolepsy and normal behavior.
Walsh, C., Beth Israel Deaconess Medical Center, HIM, Boston, Massachusetts: Genetics and molecular biology of human cerebral cortical development.

Advanced Techniques in Molecular Neuroscience

July 7–22

INSTRUCTORS
J. Boulter, University of California, Los Angeles
T. Hughes, Montana State University, Bozeman
C. Lai, Scripps Research Institute, La Jolla, California
D. Lavery, Purdue Pharma LP, Cranbury, New Jersey

ASSISTANTS
J. Arjomand, Purdue Pharma LP, Cranbury, New Jersey
I. Cheung, Scripps Research Institute, La Jolla, California
D. Hanway, Purdue Pharma LP, Cranbury, New Jersey
A. Klaassen, Interdepartmental Neuroscience Program, Los Angeles, California
W. Walwyn, University of California, Los Angeles

This newly revised laboratory and lecture course was designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the most advanced techniques in molecular neuroscience. The course curriculum was divided into three sections: an extensive and up-to-date set of laboratory exercises, daily lectures covering the theoretical and practical aspects of the various methods used in the laboratory, and a series of evening research seminars. The informal and interactive evening lectures were given by leading molecular neuroscientists and 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 (siRNA) for regulating the expression of specific genes in neurons; practical exercises in gene delivery systems including mammalian cell transfection protocols and single-cell electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use and design of 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; and methods and application of RNA amplification (aRNA). Each laboratory module was followed by comprehensive data analyses and interpretation, protocol troubleshooting, and suggestions for ways to improve or modify the existing technique. Finally, course participants were introduced to bioinformatics and a wide range of Internet resources that are available to molecular neuroscientists. This course was supported with funds provided by the National Institute of Mental Health and the Howard Hughes Medical Institute.

PARTICIPANTS

Bolduc, F., FRCPC, Cold Spring Harbor Laboratory
Brasnjo, G., B.S., University of California, Los Angeles
Drew, M., Ph.D., Columbia University Medical Center, New York
Gollan, L., Ph.D., Columbia University, New York
Gumy, L., M.S., University of Cambridge, United Kingdom
Hanson, J., Ph.D., Stanford University School of Medicine, California
Kalbaugh, T., Ph.D., National Institutes of Health/NINDS, Bethesda, Maryland
Kaselin, J., M.S., Abo Akademi University, Finland
Kreitzer, A., Ph.D., Stanford University, Palo Alto, California
McDermott, B., Ph.D., The Rockefeller University, New York
Molyneaux, B., B.A., Harvard University, Boston, Massachusetts
Rissman, E., Ph.D., University of Virginia, Charlottesville
Soviknes, A., M.S., University of Bergen, Norway
Wetherington, J., Ph.D., Emory University, Atlanta, Georgia
Yokota, Y., B.S., University of North Carolina, Chapel Hill

SEMINARS

Berlot, C., Weis Center for Research, Danville, Pennsylvania: Visualization of G-protein targeting and signaling using GFP fusions and biomolecular fluorescence complementation.
Darnell, R., The Rockefeller University, New York: Molecular techniques to dissect neurologic disease: The emerging role of RNA-binding protein in the brain.
Eberwine, J., University of Pennsylvania, Philadelphia: Neuronal dendrites: mRNA localization, translation, and some surprises.
Haas, K., University of British Columbia, Vancouver, Canada: Probing gene functions: Targeted electroporation strategies for in vivo transfection of single cells or entire tissues.
Hannon, G., Cold Spring Harbor Laboratory: RNAi: Mechanism and application.
Hughes, R., Prolexys Pharmaceuticals, Inc., Salt Lake City, Utah: Protein interactions in Huntington's disease.
Mombaerts, P., The Rockefeller University, New York: Olfaction targeted.
Tessarollo, L., National Cancer Institute, Frederick, Maryland: Dissecting neurotrophin functions in vivo: Lessons from engineered mouse models.
Walsh, C., Harvard Medical School and HHMI Beth Israel Deaconess Medical Center, Boston, Massachusetts: Mapping and cloning genes that regulate development of the human brain.
Yang, W., University of California, Los Angeles: A bacterial artificial chromosome (BAC)-mediated transgenic approach to study function and dysfunction of the mammalian brain.

Proteomics

July 9–22

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

M. Fernandez, Harvard Institute of Proteomics, Cambridge, Massachusetts
E. Hainsworth, Harvard Medical School, BCMP, Boston, Massachusetts
J. Jennings, Vanderbilt University School of Medicine, Nashville, Tennessee
T. Murthy, Harvard Medical School, Cambridge, Massachusetts
N. Ramachandran, Harvard Institute of Proteomics, Cambridge, Massachusetts
D. Veine, 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 hands-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 mapping and tandem mass spectrometry, quantification, 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 students with the fundamental knowledge and hands-on experience necessary to be able to perform and analyze proteomics experiments, and to learn to identify new opportunities in applying proteomics approaches to their own research. This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Agarwalla, S., Ph.D., University of California, San Francisco
Ambrosio, J., Ph.D., University of Mexico, Mexico City
Arnesen, T., M.S., University of Bergen, Norway
Bogusz, A., M.D., University of Chicago, Illinois
Boukh-Viner, T., B.S., Concordia University, Montreal, Canada
Castro-Rivera, E., Ph.D., University of Texas Southwestern Medical Center, Dallas
Gray, R., B.S., Michigan State University, East Lansing
Hennington, B., Ph.D., Tougaloo College, Clinton, Mississippi
Lonergan, E., Ph.D., Iowa State University, Ames
Kawaguchi, R., M.S., Penn State University, University Park
Klaper, R., Ph.D., University of Wisconsin, Milwaukee
Lenassi, M., B.S., University of Ljubljana, Slovenia
Liu, H., M.S., Massachusetts Institute of Technology, Cambridge
Moik, D., Diplom., University Clinic of Duesseldorf, Germany
Polanowska, J., Ph.D., INSERM, Marseille, France
Roux, M., B.S., Randse Afrikaanse Universiteit, Republic of South Africa

SEMINARS

Chait, B., The Rockefeller University, New York: Identifying posttranslational modifications.
Clauser, K., Broad Institute of MIT and Harvard, Cambridge, Massachusetts: Biomaker discovery and LC/MS/MS data interpretation.
Biomarker discovery and LC/MS/MS data interpretation.
Macbeath, G., Harvard University, Cambridge, Massachusetts: Applications of protein microarrays.
Pappin, D., Applied Biosystems, Foster City, California: Novel quantitative mass spectrometry approaches.
Phinney, B., Michigan State University, East Lansing: Applications of FT mass spectrometry in proteomics.
Reyzer, M., Vanderbilt University, Nashville, Tennessee: Imaging mass spectrometry.
Snyder, M., Yale University, New Haven, Connecticut: Protein microarrays.
Tempst, P., Memorial Sloan-Kettering Cancer Center, New York: Mass spectrometry and medical diagnostics.

Drosophila Genetics and Genomics

July 14–27

INSTRUCTORS **M. Ashburner,** Cambridge University and EBI, Cambridge, United Kingdom
K. Burtis, University of California, Davis
R.S. Hawley, Stowers Institute for Biomedical Research, Kansas City, Missouri

ASSISTANT **S. Langley,** University of California, Berkeley

This intensive seminar course provided an introduction to the theory and practice of methods used to analyze and manipulate the *Drosophila* genome. It is suitable for graduate students and researchers with some experience with *Drosophila* who are interested in expanding their knowledge of the wide range of genetic and genomic techniques now available for use with this organism. Topics covered included chromosome mechanics, the design and execution of genetic screens, and the use of transposable elements as genetic tools. We reviewed the current understanding of the genome sequence and discussed methodologies for genomic analysis (e.g., microarrays, gene finding methods, comparison with the sequences of related species, and the heterochromatin genomic project) and proteomics in *Drosophila*. The course was held at the Laboratory's Banbury Conference Center located on the north shore of Long Island. All participants stay within walking distance of the Center, close to tennis court, pool, and private beach.

PARTICIPANTS

Anderl, I., M.S., Umea University, Sweden
Bjorkmo, M., B.S., University of Oslo, Norway
Boundy, S., Ph.D., University of Bath, United Kingdom
Brogli, K., Diplom., ETH Zurich, Switzerland
Dasari, S., M.S., University of Kentucky, Lexington
Du, T., B.S., University of Massachusetts, Worcester
Graveley, B., Ph.D., University of Connecticut Health Center, Farmington
Haflidadottir, B., M.S., University of Iceland, Reykavik
Katsani, K., Ph.D., Institut Curie, Paris, France
Kind, J., M.S., EMBL Heidelberg, Germany
Kocks, C., Ph.D., Massachusetts General Hospital, Boston
Leinonen, A., B.S., University of Tampere, Finland
Liebert, S., M.S., Baylor College of Medicine, Houston, Texas
Lindmo, K., M.S., The Norwegian Radium Hospital, Oslo, Norway
Marconi, S., M.S., University of Bari, Italy
Mendes, C., B.S., The Rockefeller University, New York
Seppa, M., B.A., Washington University, St. Louis, Missouri
Sierralta, J., Ph.D., University of Chile, Santiago
Stier, S., M.S., Uppsala University, Sweden
Sopory, S., Ph.D., Oregon Health and Science University, Portland

SEMINARS

Baker, B., Stanford University, California: Behavioral neurogenetics in *Drosophila*.
Burtis, Kenneth, University of California, Davis: *Drosophila* genome and genomics.
Cooley, L., Yale University, New Haven, Connecticut: Fluorescent proteins in *Drosophila*: Cell biology and proteomics.
Ganetzky, B., University of Wisconsin, Madison: Segregation distortion/neurogenetics.
Golic, K., University of Utah, Salt Lake City: Mosaic systems and P-element technologies.
Hawley, R.S., Stowers Institute for Biomedical Research, Kansas City, Missouri; Ashburner, M., Cambridge University and EBI, Cambridge, United Kingdom: Introduction to *Drosophila*; Basic genetics of *Drosophila*.
Hawley, R.S., Stowers Institute for Biomedical Research, Kansas City, Missouri: Meiosis and mitosis.
Karpen, G., University of California, Berkeley: Chromosome structure.
Langley, C., University of California, Davis: Genome structure and *Drosophila* populations.
Lehman, R., New York University School of Medicine, New York: Genetic screens.
Perrimon, N., Harvard Medical School, Boston, Massachusetts: Functional genomics (RNAi).
White, K., Yale University School of Medicine, New Haven, Connecticut: Functional genomics (microarrays and network annotation).

C. elegans

July 27–August 16

INSTRUCTORS	**B. Bowerman,** University of Oregon, Eugene **S. Clark,** New York University School of Medicine, New York **M. Labouesse,** IGBMC, Strasbourg, France **I. Mori,** Nagoya University, Japan
ASSISTANTS	**C. Carter,** University of Oregon, Eugene **C. Chiu,** New York University School of Medicine, New York

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 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* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction and screening of deletion libraries, and RNA inactivation. The course was designed to impart sufficient training to students in the most important attributes of the *C. elegans* system to enable students to embark on their own research projects after returning to their home institutions. This course was supported with funds provided by the National Institute of Child Health and Human Development.

PARTICIPANTS

Beale, E., Ph.D., Texas Tech. University Health Sciences Center, Lubbock
Garrett, M., Ph.D., Vanderbilt University Medical Center, Nashville, Tennessee
Grez, P., B.S., Pontificia Universidad Catolica de Chile, Santiago
Holmes, C., B.S., University of British Columbia, Vancouver, Canada
Honda, Y., Ph.D., Tokyo Metropolitan Institute of Gerontology, Japan
Kosarek, J., B.S., University of Texas Southwestern Medical Center, Dallas
Lo, S., Ph.D., Chang Gung University, Tao Yuan, ROC
Maro, G., Ph.D., INSERM U368, Paris, France
Mertens, C., M.A., Ghent University, Belgium
Michael, M., Ph.D., Harvard University, Cambridge, Massachusetts
Nakachi, Y., M.S., Saitama Medical School, Japan
Nilsen, H., Ph.D., London Research Institutes, Herts, United Kingdom
Rockman, M., B.S., Duke University, Durham, North Carolina
Shorto, A., Ph.D., University of Bristol, United Kingdom
Simonetta, S., M.S., University of Quilmes, Buenos Aires, Argentina
Winkelbauer, M., B.S., University of Alabama, Birmingham

SEMINARS

Ewbank, J., CIML, Marseille, France: Host-pathogen interactions in *C. elegans*.
Greenwald, I., Columbia University, New York: LIN-12/Notch signaling and cell-cell interactions in *C. elegans*.
Hall, D., Einstein University School of Medicine, New York: Introduction to Worm Atlas, an anatomy database for *C. elegans* researchers.
Harris, T., Cold Spring Harbor Laboratory: WormBase: A live crash-free course, and WormBase tools for data mining and comparative genomics.
Herman, R., University of Minnesota, Minneapolis: *C. elegans* genetic suppressors, enhancers, redundancy, and mosaics.
Mango, S., University of Utah, Salt Lake City: Organogenesis and transcriptional networks in *C. elegans*.
Nonet, M., Washington University School of Medicine, St. Louis, Missouri: Regulation of synaptic transmission by Rab GTPases.
Rougvie, A., University of Minnesota, St. Paul: Keeping time with microRNAs in *C. elegans*.
Schafer, W., University of California, San Diego, La Jolla: Imaging neuronal activity and behavior in *C. elegans*.
Strome, S., Indiana University, Bloomington: Germ-line development in *C. elegans*.
Sugimoto, A., RIKEN Center for Developmental Biology, Kobe, Japan: A *C. elegans* phenome project: Toward a genomic understanding of the developmental program.

Eukaryotic Gene Expression

July 27–August 16

INSTRUCTORS

L.D. Attardi, Stanford University, California
W.L. Kraus, Cornell University, Ithaca, New York
A. Shilatifard, St. Louis University, Missouri
L. Tora, IGBMC, Illkirch, France

ASSISTANTS

T. Hilton, IGBMC, Illkirch, France
T. Johnson, Stanford University, California
M.Y. Kim, Cornell University. Ithaca, New York
A. Wood, St. Louis University Health Sciences Center, Missouri

This course was designed for students, postdocs, and principal investigators who had 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 recombinant tagged proteins, nuclear

extracts, performed in vitro transcription reactions and measured RNA levels using S1 nuclease mapping. 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, and in vivo. This included transcription assays, nucleosome mapping, chromatin remodeling assays, and restriction enzyme accessibility 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 knock-down experiments in mammalian cells. In addition, determining cellular gene expression profiles had 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. This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Binda, O., B.S., McGill University, Montreal, Canada
Fischer, A., M.S., University of Geneva, Switzerland
Heldring, N., M.S., Karolinska Institutet, Huddinge, Sweden
Iijima, K. A., Ph.D., Cold Spring Harbor Laboratory
Kent, J., Ph.D., University of California, Santa Cruz
Kodandera, D., M.S., Emory University, Atlanta, Georgia
Kotekar, A., Ph.D., National Institutes of Health, Rockville, Maryland
Lakowski, B., Ph.D, Institut Pasteur, Paris, France
Larsen, L., M.S., Steno Diabetes Center, Gentofte, Denmark
Lizcano, F., Ph.D., La Sabana University, Chia, Columbia
Lundgren, J., M.S., Stockholm University, Sweden
Magklara, A., Ph.D., National Institutes of Health/NCI, Bethesda, Maryland
Marinescu, V., Ph.D., Children's Hospital Boston, Enders, Massachusetts
Nally, K., Ph.D., National University of Ireland, Cork
Ramachandran, A., Ph.D., Tufts University School of Medicine, Boston, Massachusetts
Tan, M.-H., MRCP Van Andel Research Institute, Grand Rapids, Michigan

SEMINARS

Attardi, L., Stanford University, California: Using mouse models to dissect the function of the *p53 trans*-activator in tumor suppression.
Bartolomei, M., HHMI/University of Pennsylvania School of Medicine, Philadelphia: Regulation of genomic imprinting.
Carthew, R., Northwestern University, Evanston, Illinois: Gene silencing by small RNAs.
Conaway, J., Stowers Institute for Medical Research, Kansas City, Missouri: Regulational of initiation and elongation by RNA polymerase II.
Cote, J., Laval University Cancer Research Center, Quebec City, Canada: Chromatin modification by the NuA4 complex: An early step in gene activation.
Dynlacht, B., New York University School of Medicine, New York: Transcriptional control of the cell cycle.
Fuller, M., Stanford University School of Medicine, California: Developmental regulation of gene expression by tissue-specific transcription machinery.
Gill, G., Harvard Medical School, Boston, Massachusetts: Regulation of transcription factor activity by SUMO modification.
Graves, B., University of Utah, Salt Lake City: The ETS family of transcription factors: Routes to specificity.
Kraus, W. L., Cornell University, Ithaca, New York: Role of ligands, coactivators, and chromatin in estrogen-receptor-dependent transcription.
Nyborg, J., Colorado State University, Fort Collins: Regulation of HTLV-I transcription in a chromatin context.
Sassone-Corsi, P., Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France: Unique chromatin remodeling and transcriptional rules in male germ cells.
Schultz, P., Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkrich, France: Structural studies of large transcription complexes of electron microscopy and image processing.
Shilatifard, A., St. Louis University School of Medicine, Missouri: A COMPASD and a GPS in defining histone modifications, transcriptional regulation, and human cancer: The coordinates of the genome.
Timmers, M., University Medical Centre, Utrecht, The Netherlands: Dynamic regulation of transcription by RNA polymerase II.
Tora, L., Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkrich, France: Different sets of promoter recognition factors regulate the transcription initiation of RNA polymerase II at distinct promoters.
Whitfield, M., Dartmouth Medical School, Hanover, New Hampshire: Genome-wide analysis of gene expression in basic biology and complex human disease.
Workman, J., Stowers Institute for Medical Research, Kansas City, Missouri: Protein complexes that modify chromatin.

Imaging Structure and Function in the Nervous System

July 27–August 16

INSTRUCTORS

F. Engert, Harvard University, Cambridge, Massachusetts
F. Helmchen, Max-Planck Institut für Medizinische Forschung, Heidelberg, Germany
D. Kleinfeld, University of California, San Diego, La Jolla
T. Murphy, University of British Columbia, Vancouver, Canada
M. Oheim, Ecole Superieure de Physique et Chimie Industrielles, Paris, France

ASSISTANTS

V. Emiliani, Institut Jacques Monod, Paris, France
T. Nevian, Max-Planck Institut für Medizinische Forschung, Heidelberg, Germany
Y. Otsu, University of British Columbia, Vancouver, Canada
P. Tsai, University of California, San Diego, La Jolla
W. Tyler, Harvard University, Cambridge, Massachusetts
R. Vislay, Harvard University, Cambridge, Massachusetts
J. Wadiche, Vollum Institute, Portland, Oregon
J. Waters, Max-Planck Institut, Heidelberg, Germany

Advances in light microscopy and digital image processing, and the development of a variety of powerful fluorescent probes, presented 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 pri-

mary 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, photo-activated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multi-photon laser-scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. We used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells. Applicants had a strong background in the neurosciences or in cell biology. This course was supported with funds provided by the National Institute of Mental Health, the National Institute on Drug Abuse, and the Howard Hughes Medical Institute

PARTICIPANTS

Awatramani, G., Ph.D., OSHU/Vollum Institute, Portland, Oregon
Beckman, M., Ph.D., Yale University/The University of Montana, New Haven, Connecticut
Bezzi, P., Ph.D., University of Lausanne, Switzerland
Brasnjo, G., B.S., University of California, Los Angeles
Dan, C., Ph.D., Harvard University, Cambridge, Massachusetts
Khosravani, H., M.S., University of Calgary, Canada
Komiyama, T., B.S., Stanford University, Palo Alto, California
Maggi, L., Ph.D., EMBL-Monterotondo, Roma, Italy
Mrsic-Flogel, T., Ph.D., Max-Planck Institute of Neurobiology, Martinsried, Germany
Pawlu, C., M.D., University of Freiburg, Germany
Topolnik, L., Ph.D., University of Montreal, Canada
Tsui, J., B.A., Stanford University, Palo Alto, California

SEMINARS

Almers, W., Oregon Health and Science University, Portland: TIRF.
Betz, W., University of Colorado Medical School, Denver: FM143.
Cohen, L., Yale University, New Haven, Connecticut: Voltage sensitive dyes.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: Block-face neuronal reconstruction.
Engert, F., Harvard University, Cambridge, Massachusetts: Loading in the tadpole (introduction of a basic prep).
Glaser, J., Microbrightfield, Inc.: Reconstruction of neurons.
Helmchen, F., Max-Planck Institut für Medizinische Forschung, Heidelberg, Germany: Ionic indicators/buffering/basics.
Hu, C.-D., Purdue University, West Lafayette, Indiana: Seeing is believing: Visualization of transcription factor interactions...using bimolecular fluorescence complementation.
Kalatsky, V., University of California, San Francisco: Intrinsic imaging.
Keller, E., Carl Zeiss, Inc., Thornwood, New York: Diffraction theory on image formation.
Kleinfeld, D., University of California, San Diego, La Jolla: Basic optics.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Point spread functions.
Lichtmann, J., Washington University, St. Louis, Missouri: Confocal microscopy.
Mertz, J., Ecole Superieure de Physique et Chimie Industrielles, Paris, France: Advances in nonlinear microscopy.
Moomaw, B., Spring Branch, Texas: CCD imaging, principles, and technology.
Murphy, T., University of British Columbia, Vancouver, Canada: Back to basics, experimental design, issues with live cell imaging.
Oheim, M., Ecole Superieure de Physique et Chimie Industrielles, Paris, France: Basics of TIRF microscopy.
Shepherd, G., Cold Spring Harbor Laboratory: Caged transmitters and circuit analysis.
Svoboda, K., Cold Spring Harbor Laboratory: Basics of 2-photon microscopy.
Tsien, R., University of California, San Diego, La Jolla: The world of functional XFPs.

Yeast Genetics

July 27–August 16

INSTRUCTORS
D. Amberg, SUNY, Syracuse, New York
D. Burke, University of Virginia, Charlottesville
J. Strathern, National Cancer Institute, Frederick, Maryland

ASSISTANTS
C. Copeland, University of Virginia, Charlottesville
M. Derbyshire, National Center for Biotechnology, National Institutes of Health, Bethesda, Maryland
M. Farah, SUNY Upstate Medical University, Syracuse, New York
B. Keyes, University of Virginia, Charlottesville
F. Malagon, National Cancer Institute, Frederick, Maryland

This modern, state-of-the-art laboratory course is designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches are emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques, including various types of yeast transformation, gene replacement with plasmids and 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 course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Arnason, E., Ph.D., University of Iceland, Reykjavik
Brady, T., B.S., Iowa State University, Ames
Bulteau, A. L., Ph.D., Case Western Reserve University, Cleveland, Ohio
Chabes, A. L., Ph.D., Cold Spring Harbor Laboratory
Charvin, G., B.S., The Rockefeller University, New York
Garbitt, R., B.S., B.A., Tufts University, Boston, Massachusetts
Gresham, D., Ph.D., Princeton University, New Jersey
Magwene, P., Ph.D., University of Pennsylvania, Philadelphia
Mercer, J., Ph.D., Duke University, Durham, North Carolina
Paumi, C., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Saerens, S., M.S., Catholic University of Leuven, Belgium
Sandberg, R., M.S., Karolinska Institutet, Stockholm, Sweden
Sengpta, A., Ph.D., Rutgers University, Piscataway, New Jersey
Tong, Z., B.S., University of Pennsylvania, Philadelphia
Tseng, C., A.B., Harvard Medical School, Boston, Massachusetts
Wagner, M., B.S., University of California, San Diego, La Jolla

SEMINARS

Basrai, M., National Cancer Institute, Bethesda, Maryland: Molecular determinants of faithful chromosome transmission and checkpoint regulation.
Boone, C., University of Toronto, Ontario, Canada: Global mapping of the yeast genetic interaction network.
Botstein, D., Princeton University, New Jersey: Functional genomics: Getting beyond descriptive studies.
Bretscher, A., Cornell University, Ithaca, New York: Combining genetics and cell biology to elucidate the mechanism of polarized growth and organelle segregation.
Curcio, J., University at Albany School of Public Health, New York: Retrotransposons to the rescue: How Ty1 elements respond to challenges to the genome.
Emr, S., HHMI/University of California, San Diego: From genes to functions: Genetics of phosphoinositide lipid and ubiquitin signaling in receptor sorting at the endosome.
Johnston, M., Washington University School of Medicine, St. Louis, Missouri: Feasting, fasting and fermenting: Glucose sensing and signaling in yeasts.
Konopka, J., Stony Brook University, New York: Genetic analysis of the pheromone pathway: The meaning of screening and the basis for epistasis.
Michaelis, S., Johns Hopkins School of Medicine, Baltimore, Maryland: Biogenesis of the a-Factor mating pheromone in *S. cerevisiae*: From yeast mating to human disease.
Michels, C., Queens College/City University of New York, Flushing: The regulation of maltose fermentation: A complex brew.
Pringle, J., University of North Carolina, Chapel Hill: Morphogenesis and cytokinesis in yeast.
Smith, M.M., University of Virginia, Charlottesville: Genetics of chromatin structure and function.
Stearns, T., Stanford University, California: Yeast genetics: Teaching it and doing it—at the same time!
Tyers, M., Samuel Lunenfeld Research Institute/Mt. Sinai Hospital, Toronto, Canada: Genetics of size control: From the classic to the systematic.

Stem Cells

July 29–August 11

INSTRUCTORS
G. Keller, Mt. Sinai School of Medicine, New York
R. McKay, National Institutes of Health, Bethesda, Maryland
A. McLaren, University of Cambridge, United Kingdom
J. Rossant, Samuel Lunenfeld Research Institute, Toronto, Canada
A. Surani, University of Cambridge, United Kingdom

This two-week lecture course brought together leading researchers in the stem cell field with a small group of international students. Stem cells construct organs in development. They sustain tissues in the adult and restore them after injury. Because of these properties, isolating and manipulating stem cells has become a major new element in biomedical science. This lecture and discussion course covered a series of subjects including the cells of the early embryo, the nature of germ cells, the mechanisms that control the number of stem cells, and their stability and transformation into other cell types. The clinical potential and political impact of stem cell technology was also presented in depth by invited speakers. A key feature of the course was the easy access to the instructors and the invited lecturers for informal discussion. The purpose of the course provided participants with an opportunity to achieve an advanced understanding of the scientific and clinical importance of stem cells. The course was held at the Laboratory's Banbury Conference Center located on the north shore of Long Island. All participants stayed within walking distance of the Center, close to tennis court, pool, and private beach. This is a unique learning environment. Partial support for the course was provided by the Eppley Foundation for Research and the Howard Hughes Medical Institute.

PARTICIPANTS

Abeyewickreme, A., B.A., Institute of Child Health, London, United Kingdom
Bao, J., Ph.D., Washington University, St. Louis, Missouri
Bian, Z., Ph.D., Karolinska Institutet, Stockholm, Sweden
Erices, A., Ph.D., Universidad Andres Bello, Santiago, Chile
Fossati, V., Ph.D., University of Bologna, Italy
Gangemi, R., M.D., National Institute of Cancer Research, Genova, Italy
Hipp, J., B.S., Wake Forest University School of Medicine, Winston-Salem, North Carolina
Lin, S.-Y., Ph.D., Mackay Memorial Hospital, Taipei, Taiwan
Maeda, T., B.E., Osaka University, Japan
Mantle, I., Ph.D., University of Michigan, Ann Arbor
Newey, S., Ph.D., Cold Spring Harbor Laboratory
Pillai, A., B.S., University of California, San Diego, La Jolla
Riva, F., Ph.D., University of Pavia, Italy
Rochford, C., B.S., European Neuroscience Institute, Gottingen, Germany
Schenke-Layland, K., Ph.D., Friedrich Schiller University, Jena, Germany
Schmelzer, E., Ph.D., University of North Carolina, Chapel Hill
Tesar, P., Ph.D., National Institutes of Health and University of Oxford, Bethesda, Maryland
Testa, G., Ph.D., Dresden University of Technology/Max-Planck Institute, Germany
Wahle, J., B.S., University of South Florida, Tampa
Wirta, V., M.S., Royal Institute of Technology, Stockholm, Sweden

SEMINARS

Battey, J., National Institutes of Health/NIDCD, Bethesda, Maryland: Politics of stem cells.
Burden, S., New York University School of Medicine, New York: Muscle function and regeneration.
Coffey, R., Vanderbilt University School of Medicine, Nashville, Tennessee: Colon cancer.
Eidelberg, D., North Shore–Long Island Jewish Health System, Manhasset, New York: Cell therapy in Parkinson's disease.
Geijsen, N., Massachusetts General Hospital, Charlestown: Derivation of germ cells from ES cells.
Gould, E., Princeton University, New Jersey: Neurogensis in the adult brain.
Johnston, L., Columbia University, New York: Control of cell growth and cell division during development.
Keller, G., Mt. Sinai School of Medicine, New York: Hematopoiesis by ES cells.
Kikyo, N., University of Minnesota, Minneapolis: Genomic reprogramming.
Krause, D., Yale University, New Haven, Connecticut: Transdifferentiation of bone marrow.
Lehmann, R., Skirball Institute, New York University, New York: *Drosophila* germ cells.
Lemischka, I., Princeton University, Rutgers, New Jersey: Transcriptional signature of stem cells.
Mandel, G., Stony Brook University, New York: Mechanisms regulating neuronal phenotype and behavior.
McKay, R., National Institutes of Health/NINDS, Bethesda, Maryland: Neural stem cells.
Schier, A., Skirball Institute of Biomolecular Medicine, New York: Nodal signaling in vertebrate development.
Simmons, P., Peter MacCallum Cancer Institute, Australia: Cells in the bone marrow.
Smith, A., Institute for Stem Cell Research, United Kingdom: Embryonic stem cells.
Snoeck, H., Mt. Sinai School of Medicine, New York: Genetics of hematopoiesis.
Tam, P., University of Sydney, Australia: Mechanisms of tissue patterning.
Trumpp, A., Swiss Institute for Experimental Cancer Research, Switzerland: Development and tumorigenesis.
van Lohuizen, M., Netherlands Cancer Institute, The Netherlands: Epigenetic regulation of transcriptional states by higher-order chromatin structures.
Wicha, M., University of Michigan Comprehensive Cancer Center, Ann Arbor: Breast cancer.
Zoloth, L., Northwestern University, Evanston, Illinois: Stem cell ethics.

X-ray Methods in Structural Biology

October 18–November 2

INSTRUCTORS

W. Furey, V.A. Medical Center, Pittsburgh, Pennsylvania
G. Gilliland, National Institute of Standards & Technology, Gaithersburg, Maryland
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku/MSC, Inc., The Woodlands, Texas

ASSISTANT

I. Persakova, Rutgers, The State University of New Jersey, New Brunswick

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensive laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics covered included basic diffraction theory, crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction, molecular replacement, solvent flattening, noncrystallographic symmetry averaging, electron density interpretation, structure refinement, structure validation, molecular graphics, and coordinate deposi-

tion. Participants learned through extensive hands-on experiments and formal presentations. They crystallized, determined, and refined a protein structure in parallel with lectures on the theory and informal discussions behind the techniques. Extensive use of Linux workstations was made throughout the course for computational aspects of the process. This course was supported by the National Cancer Institute.

PARTICIPANTS

Beglova, N., Brigham & Women's Hospital, Boston, Massachusetts
Cochrane, J., Yale University, New Haven, Connecticut
Dombrauckas, J., University of Illinois, Chicago
Dzivenu, O., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Kamenski, T., University of Munich, Germany
Kawate, T., Columbia University, New York
Lee, M., University of Sydney, Australia
Litowski, J., Ph.D., Columbia University, New York
Loukachevitch, L., Vanderbilt University School of Medicine, Nashville, Tennessee
Mylona, A., European Molecular Biology Laboratory, Grenoble, France
Rhodes, W., University of Southern Maine, Portland
Sivakumar, N., Institute of Molecular Agrobiology, Singapore
Wan, Q., University of Vermont, Burlington
Wei, Y., Brookhaven National Laboratory, Upton, New York
Wilson, K.-A., The Rockefeller University, New York
Yip, C., University of British Columbia, Vancouver, Canada

SEMINARS

Dauter, Z., Brookhaven National Laboratory, Upton, New York: Anomalous data collection.
Gilliland, G., National Institute of Standards and Technology, Gaithersburg, Maryland: The protein data bank.
Hendrickson, W., Columbia University, New York: MAD phasing: Theory and practice.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Argonaute: The secret of Slicer.
Kjeldgaard, M., Aarhus University, Denmark: Electron density fitting from A to 0.
Kleywegt, G., University of Uppsala, Sweden: Just because it's in *Nature*, doesn't mean it's true...(macromolecular structure validation).
Perrakis, A., Netherlands Cancer Institute, Amsterdam, The Netherlands: Shaping the human genome: Structural insights on the action of retrotransposons.
Pflugrath, J., Rigaku/MSC, Inc., The Woodlands, Texas: Away from the edge: Sulfur SAD with chromium radiation.
Richardson, D., Duke University Medical Center, Durham, North Carolina; Richardson, J., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using all atom contacts.
Strobel, S., Yale University, New Haven, Connecticut: RNA crystallography: Structure of a self-splicing intron reaction intermediate.
Terwilliger, T., Los Alamos National Laboratory, New Mexico: Solve and resolve.

Bioinformatics: Writing Software for Genome Research

October 20–November 2

INSTRUCTORS
S. Lewis, University of California Berkeley
S. Prochnik, University of California, Berkeley
L. Stein, Cold Spring Harbor Laboratory
J. Tisdall, DuPont Experimental Station, Wilmington, Delaware

ASSISTANTS
J. Aerts, Wageningen University, The Netherlands
S. Cain, Cold Spring Harbor Laboratory
K. Clark, Cold Spring Harbor Laboratory
M. Dybbs, University of California, Berkeley
R. Halgren, Michigan State University, East Lansing
G. Hartzell, University of California, Berkeley
C. Kennedy, University of California, Berkeley
B. O'Connor, University of California, Los Angeles
J. Stein, Cereon Genomics, Cambridge, Massachusetts
C. Yamada, University of Cambridge, United Kingdom

The desktop computer has rapidly become an indispensable tool in the biologist's tool chest. The success of the human genome project created an explosion of information: billions of bits of biological information stashed electronically in databases around the globe just waiting for the right key to unlock them. New technologies such as DNA microarrays and high-throughput genotyping are creating an information overload that the traditional laboratory notebook cannot handle. To exploit the information revolution in biology, biologists moved beyond canned Web interfaces and Excel spreadsheets. They

took charge of the data by creating their own software to fetch, manage, and integrate it. The goal of this course was to provide biologists with the tools needed to deal with this changing landscape. Designed for students and researchers with little prior knowledge of programming, this two-week course taught the fundamentals of the Unix operating system, Perl scripting, dynamic Web page development with the CGI protocol, and database design. The course combined formal lectures with hands-on experience in which students worked to solve a series of problem sets drawn from common scenarios in biological data acquisition, integration, and laboratory workflow management. For their final projects, students were posed problems using their own data and worked with each other and the faculty to solve them. Note that the primary focus of this course provided students with the practical aspects of software development, rather than presenting a detailed description of the algorithms used in computational biology. For the latter, we recommend the Computational Genomics course. This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Acosta, I., B.S., Yale University, New Haven, Connecticut
Chalkia, D., M.S., Penn State University, University Park
Crayton, III, M., Ph.D., University of North Carolina, Chapel Hill
Emes, R., Ph.D., University College London, United Kingdom
Gillespie, M., Ph.D., Cold Spring Harbor Laboratory
Gopinathrao, G., Ph.D., Cold Spring Harbor Laboratory
Goto, D., Ph.D., Cold Spring Harbor Laboratory
Hsiao, W., Ph.D., Simon Fraser University, Burnaby, Canada
Hurle, B., Ph.D., National Human Genome Research Institute, NIH, Bethesda, Maryland
Karalius, J., B.S., University of Cambridge, United Kingdom
Mapelli, M., Ph.D., European Institute of Oncology, Milan, Italy
Marques, T., Ph.D., Universitat Pompeu Fabra, Barcelona, Spain
Mund, C., B.S., German Cancer Research Center, Heidelberg, Germany
Nikolaidis, N., Ph.D., Penn State University, University Park
Przeworski, M., Ph.D., Brown University, Providence, Rhode Island
Samuelson, A., Ph.D., Massachusetts General Hospital, Boston
Tammoja, K., M.S., National Institute for Health Development, Tallinn, Estonia
Yi, S., Ph.D., Georgia Institute of Technology, Atlanta, Georgia

SEMINARS

Ashburner, M., University of Cambridge, United Kingdom: Biological oncologies.
Brent, M., Washington University, St. Louis, Missouri: Gene prediction.
Hartzell, G., University of California, Berkeley: The tsao of perl.
Marth, G., Boston College, Chestnut Hill, Massachusetts: Sequence variation analysis.
Pearson, W., University of Virginia, Charlottesville: Sequence similarity analysis.
Peitzsch, R., Pfizer Global R&D, Groton, Connecticut: Modeling biological data in relational databases.
Stein, L., Cold Spring Harbor Laboratory: Generic genome browser.
Troyanskay, O., Princeton University, New Jersey: Mircroarray analysis.
Yandell, M., HHMI/University of California, Berkeley: The BLAST algorithm; Interpreting BLAST statistics.

Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 20–November 2

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 **T. Howard,** University of New Mexico, Albuquerque
K. Hu, Scripps Research Institute, La Jolla, California
W. Moore, The University of Dundee, United Kingdom
J. Peng, University of California, Berkeley
M. Platani, EMBL, Heidelberg, Germany

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 techniques 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 also 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 presented up-to-the-minute reports on current methods and research using the techniques being presented. This course was supported with funds provided by the National Cancer Institute

PARTICIPANTS

Bourke, S., B.S., University of Leeds, United Kingdom
Demarco, I., M.S., University of Virginia, Charlottesville
Fehrenbacher, N., M.A., Danish Cancer Society, Copenhagen, Denmark
Gallegos, M., Ph.D., University of California, San Francisco
Ivakhno, S., B.S., Institute of Molecular Biology and Genetics, Kyiv, Ukraine
Larsen, L., M.S., Steno Diabetes Center, Gentofte, Denmark
Manning, L., B.S., University of Pittsburgh, Pennsylvania
Novak, I., B.S., Karolinska Institutet, Stockholm, Sweden
Psatha, M., Ph.D., Brigham & Women's Hospital, Boston, Massachusetts
Pugacheva, E., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Schauss, A., Diplom., Max-Planck Institute for Biophysical Chemistry, Goettingen, Germany
Specktor, A., B.A., New York University School of Medicine, New York
Steindler, L., M.S., Tel Aviv University, Israel
Stephano, J., Ph.D., Universidad Autonoma de Baja California, Ensenada, Mexico
Wang, H., Ph.D., University of Massachusetts Medical School, Worcester
Wang, L., Ph.D., University of Pittsburgh, Pennsylvania

SEMINARS

Day, R., University of Virginia, Charlottesville: Seeing colors: Applications and limitations of the fluorescent proteins.
Dernburg, A., Lawrence Berkeley National Laboratory, Berkeley, California: Basics of DNA FISH.
Difilippantonio, M., National Cancer Institute/NIH, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Howard, T., University of New Mexico, Albuquerque: Basics of RNA FISH.
Hu, K., Scripps Research Institute, La Jolla, California: Basic introduction to light and fluorescence microscopy.
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.
Spector, D., Cold Spring Harbor Laboratory: Localization of gene expression by FISH and in living cells.
Stout, A., University of Pennsylvania, Philadelphia: TIRF microscopy: Implementation and application to live-cell imaging.
Svoboda, K., Cold Spring Harbor Laboratory: Applications of Z-photon microscopy to imaging in vivo.
Tran, P., University of Pennsylvania, Philadelphia: Cameras and digital imaging fundamentals.
Waterman-Storer, C., Scripps Research Institute, La Jolla, California: Quantitative fluorescent speckle microscopy in studies of cell migration.

Computational Genomics

November 3–9

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; students were encouraged to pose challenging sequence analysis problems using their own data. The course made extensive use of local WWW pages to present problem sets and the computing tools to solve them. Students used Windows and Mac workstations attached to a Unix server; participants should be comfortable using the Unix operating system and a Unix text editor. The course was designed for biologists seeking advanced training in biological sequence analysis, computational biology core resource directors and staff, and

scientists in other disciplines, such as computer science, who wish to survey current research problems in biological sequence analysis. The primary focus of this course was the theory and practice of algorithms used in computational biology, with the goal of using current methods more effectively and developing new algorithms. Students more interested in the practical aspects of the advanced software development were encouraged to apply to course on Advanced Bioinformatics. This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Abernathy, B., B.S., Purdue University, West Lafayette, Indiana
Almedia, P., B.S., Instituto Gulbenkian de Ciencia, Oeiras, Portugal
Bai, Y., M.S., University of Texas, Arlington
Boukhgalter, B., Ph.D., Broad/MIT Institute for Genome Research, Cambridge, Massachusetts
Dimitrova, N., Ph.D., Cold Spring Harbor Laboratory
Hagen, D., B.S., Texas A&M University, College Station
Hecht, S., Ph.D., Grand Valley State University, Allendale, Michigan
Hladish, T., B.S., University of Maryland Biotechnology Institute, Rockville
Horth, L., Ph.D., University of California, Davis
Huebinger, R., M.S., Texas A&M University, College Station
Hung, L.-W., Los Alamos National Laboratory, Berkeley, California
Kelley, J., B.S., University of Cambridge, United Kingdom
Khatun, J., Ph.D., University of North Carolina, Chapel Hill
Proctor, R., Ph.D., NCAUR-Agriculture Research Service-USDA, Peoria, Illinois
Rainey, S., Ph.D., Queen's University Belfast, North Ireland
Ren, X.-Y., M.S., Wageningen University and Research Center, The Netherlands
Robertson, C., Ph.D., Geospiza, Inc., Seattle, Washington
Sequin, A., Ph.D., Natural Resources Canada, Sainte-Foy, Quebec
Shanks, O., Ph.D., U.S. Environmental Protection Agency, Cincinnati, Ohio
Vinayaka, C., Ph.D., National Biomedical Research Foundation, Washington, D.C.

SEMINARS

Altschul, S., National Center for Biotechnology Information, Bethesda, Maryland: Statistics of sequence similarity scores; Iterated protein database searches with PSI-BLAST.
Birney, E., EMBL-EBI, Hinxton, United Kingdom: The ENSEMBL genome database; Genome computation II–ensmart.
Cooper, P., National Center for Biotechnology Information, National Library of Medicine, Bethesda, Maryland: NCBI genome resources.
Rubin, E., Joint Genome Institute, Berkeley, California: Comparative genomics and biology.
Yandell, M., HHMI/University of California, Berkeley: Genome annotation; Large-scale computation on genomes–1.

Phage Display of Proteins and Peptides

November 9–22

INSTRUCTORS **C. Barbas,** Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, University of California, La Jolla

ASSISTANTS **K. Noren,** New England Biolabs, Beverly, Massachussetts
J. Struthers, University of California, San Diego
C. Tuckey, New England Biolabs, Beverly, Massachussetts

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 covered. Peptide phage libraries were also selected 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. This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Daad, G.A., M.S., Texas A&M University, College Station
Aggarwal, S., M.S., The Johns Hopkins University, Baltimore, Maryland
Alm, T., M.S., Royal Institute of Technology, Stockholm, Sweden
Barman, I., M.A., University of California, San Francisco
Bosnar, M., B.S., Proizvodnja lijekova i vakcina–Research Institute, Ltd., Zagreb, Croatia
Bratkovic, T., B.S., Lek Pharmaceutical Company, Ljubljiana, Slovenia
Friedman, M., Ph.D., Royal Institute of Technology, Stockholm, Sweden
Grippo, V., M.S., Institute of Genetics Engineering and Molecular Biology Research, Buenos Aires, Argentina
Gronwall, C., M.S., Royal Institute of Technology, Stockholm, Sweden
Lee, S., Ph.D., Singapore General Hospital, Singapore
Love, K., B.S., Harvard Medical School, Boston, Massachusetts
Mahajan, S., Ph.D., Paradigm Pharmaceuticals, Roseville, Minnesota
Mason, P., B.Sc., Shriners Hospital/McGill University, Montreal, Quebec, Canada
Mir, S., M.S., Max-Planck Institute for Biophysics, Frankfurt Am Main, Germany
Ramasoota, P., Ph.D., Mahidol University, Bangkok, Thailand
Sato, S., B.A., Harvard Medical School, Southborough, Massachusetts

SEMINARS

Barbas, C., 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: Immunobiology of B cell and antibody responses.
Lowman, H., Genentech, Inc., San Francisco, California: SAR of peptides using phage.
Noren, C., New England Biolabs, Beverly, Massachusetts: Phage peptide libraries: The PhD for peptides.
Sidho, S., Genentech, 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: Selection of complex human antibody libraries.
Smith, G., University of Missouri, Columbia: Phage display of peptides.
Wilson, I., 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

May 4–5, August 31–September 1, December 1–2

TRAINERS
J. Gergel, Cold Spring Harbor Laboratory
G. Howell, The Jackson Laboratory
P. Rabinowicz, The Institute for Genomic Research

Initiated in 2002, this course is an intensive two-day introduction to bioinformatics that was held three times in 2004 and trained 73 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 at Woodbury located seven miles south of the main Laboratory campus. Each student was provided with a PC laptop with wireless modem for the duration of the course. Students were encouraged to supply problem sets and sequences of interest to the trainers for possible incorporation as examples in the modules. Materials were made available on the Web and students continued to ask questions of the trainers as they applied what they had learned in their individual endeavors.

May 4–5

Aho, E,. Ph.D., Concordia College, Moorhead, Minnesota
Basu, C., Ph.D., University of Tennessee, Knoxville
Blander, J., Ph.D. Yale University School of Medicine, New Haven, Connecticut
Cameron, P., Ph.D., University of Illinois, Chicago
Chun, Y.H., Ph.D., University of Michigan, Ann Arbor
Das, S., Ph.D., University of Nebraska Medical Center, Omaha
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Lysakowski, A., University of Illinois, Chicago
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Nielsen, A., Ph.D., Novozymes A/S, Bagsvaerd, Denmark
Pirity, M., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Pittenger Alley, L., B.S., USDA-ARS, Athens, Georgia
Reeleder, R., Ph.D., Agriculture and Agri-Food Canada, London, England
Snyder, D., B.S., Yale University, New Haven, Connecticut
Tan, M.-H., Ph.D., Van Andel Research Institute, Grand Rapids, Michigan
Ungvari, Z., Ph.D., New York Medical College, Valhalla
Vinayaka, C., Ph.D., National Biomedical Research Foundation, Washington, D.C.
Wellen, K., B.S., Harvard School of Public Health, Boston, Massachusetts
Wong, A., B.S., University of California, Davis
Zhao, Z., Ph.D., University of Michigan, Ann Arbor

August 31–September 1

Bazinet, C., Ph.D., St. John's University, Jamaica, New York
Bowman, M., B.S., Lancaster Labs, Richmond, Virginia
Brundage, J., B.S., Northwestern University, Evanston, Illinois
Chen, J., Ph.D., Mt. Sinai School of Medicine, New York
Chugh, S., Ph.D., Oregon Health & Science University, Portland
Cohen, P., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Glynn, N., Ph.D., USDA-ARS, Canal Point, Florida
Guo, X., Ph.D., Cold Spring Harbor Laboratory
Guris, D., B.S., University of Chicago, Illinois
Kennerson, M., Ph.D., Neurobiology Laboratory ANZAC Research Institute, Concord, New South Wales, Australia
Kutty Selva, N., Ph.D., Lund University, Sweden
Latkany, P., Ph.D., New York Eye and Ear Infirmary, New York
Lee, K., B.S., Baylor College of Medicine, Houston, Texas
Lee, Y., Ph.D., Tufts University, Boston, Massachusetts
MacMillan, T., B.S., Defence R&D Canada–Suffield Medicine Hat, Alberta, Canada
McMahon, A., Ph.D., Harvard University, Cambridge, Massachusetts
Noehammer, C., Ph.D., ARC Seibersdorf Research GmbH, Seibersdorf, Austria
Ren, L., Ph.D., Cold Spring Harbor Laboratory
Ridley, S., Ph.D., National Science Foundation, Arlington, Virginia
Serspinski, T., B.S., Fairleigh Dickinson University, Edgewater, New Jersey
Shoemaker, K., Ph.D., ZymoGenetics, Seattle, Washington
Svetlanov, A., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Ward, J., Ph.D., Middlebury College, Vermont
Witonsky, P., Ph.D., Retired Professor: West Chester University, Cambridge, Massachusetts
Wong, D., Ph.D., Western Regional Research Center, USDA–ARS, Albany, California

December 1–2

Avila, A., National University of Mexico–Nitrogen Fixiation, Cuernavaca
Boczek, L., B.S., U.S. Environmental Protection Agency, Cincinnati, Ohio
Calie, P., Ph.D., Eastern Kentucky University, Richmond
Chen, L., B.S., ZymoGenetics, Seattle, Washington
Csiszar, A., Ph.D., New York Medical College, Valhalla
Dominguez Vidana, R., B.S., UNAM, Cuernavaca, Mexico
Fisher, G., Ph.D., Ridgefield High School, Connecticut
Gonzaga-Jauregui, C., B.S., National University of Mexico, Mexico City
Griffis, J., B.S., Pioneer Hi-Bred Inc., International, Johnston, Iowa
Jobanputra, V., Ph.D., Columbia University, New York
Kelty, C., B.S., USEPA, Cincinnati, Ohio
Levon, K., Ph.D., Polytechnic University, Brooklyn, New York
Li, G., Ph.D.,The University of Hong Kong, Hong Kong
Mesri, E., Ph.D., Weill Cornell Medical College, New York
Morales, L., B.S., National University of Mexico–Nitrogen Fixiation, Cuernavaca
Singh, C., B.S., West Chester University of Pennsylvania, West Chester
Song, J.-J., B.S., Cold Spring Harbor Laboratory
Thiagarajan, R., B.S., Transgenomic, Gaithersburg, Maryland
Yang, Y., Ph.D., GlaxoSmithKline Pharmaceuticals, King of Prussia, Pennsylvania
Zheng, X., Ph.D., Children's Hospital of Philadelphia, Pennsylvania

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SEMINARS

INVITED SPEAKER PROGRAM

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their findings on a weekly basis. These seminars keep the CSHL staff current on the latest developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

JANUARY

Dr. Frank Rauscher III, The Wistar Institute. Coordinating histone modification, HP1 deposition, and DNA methylation: A model for gene variegation at euchromatic loci in mammalian cells. (Host: Winship Herr)

Dr. Ray Deshaies, Division of Biology and HHMI, California Institute of Technology. Mechanism and regulation of the SCF ubiquitin ligase-proteasome pathway. (Host: William Tansey)

FEBRUARY

Dr. David Haig, Department of Organismic and Evolutionary Biology, Harvard University. Broken symmetries: Genomic imprinting and social behavior. (Host: Dmitri Chklovskii)

Dr. John Allman, California Institute of Technology. The spindle neurons of fronto-insular and anterior cingulate cortex: A phylogenetic specialization of apes and humans related to social cognition. (Host: Dmitri Chklovskii)

Dr. Zena Werb, Department of Anatomy, University of California, San Francisco. The tumor microenvironment: Friend or foe? (Host: Senthil Muthuswamy)

MARCH

Dr. Ulrike Heberlein, Department of Anatomy, University of California, San Francisco. Studying addiction in *Drosophila*. (Host: Hollis Cline)

Dr. Roger Nicoll, University of California, San Francisco. Glutamate receptor trafficking and synaptic plasticity. (Host: Roberto Malinow)

Dr. Pamela Silver, Dana Farber Cancer Institute, Harvard Medical School. Connecting the genome to the cytoplasm. (Host: Adrian Krainer)

APRIL

Dr. Michael Rosbash, Department of Biology, HHMI/ Brandeis University. Circadian rhythms in flies: Molecules, cells, systems, and behavior. (Host: Josh Huang)

Dr. Stanislas Leibler, The Rockefeller University. Fluctuations, noise, and phenotypic variability. (Host: Yuri Lazebnik)

OCTOBER

Dr. Alan Hall, MRC Laboratory for Molecular Cell Biology and Cell Biology Unit, University College London. Rho GTPases and the control of directed cell migration. (Host: Linda Van Aelst)

NOVEMBER

Dr. Ann-Shyn Chiang, Brain Research Center, National Tsing Hua University, Taiwan. Mapping candidate memory circuits in the brain. (Host: Tim Tully)

Dr. Chris Walsh, Howard Hughes Medical Institute, Harvard Medical School. Genes that control the shape and size of the human cerebral cortex. (Host: Grigori Enikolopov)

DECEMBER

Dr. Liqun Luo, Stanford University, Department of Biological Sciences. Exploring neural circuits using genetic mosaic methods in flies and mice. (Host: Linda Van Aelst)

Dr. Danesh Moazed, Department of Cell Biology, Harvard Medical School. Targeting RNAi to the chromosome. (Host: William Tansey)

IN-HOUSE SEMINAR PROGRAM

Cold Spring Harbor In-House Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

JANUARY

Lincoln Stein: A tale of two worms: The *C. briggsae* sequencing project.
Eve-Ellen Govek (Van Aelst Lab): The role of Rho-linked MRX genes in neuronal morphogenesis.
Carlos Brody: A neural model of sequential discrimination: Memory loading, memory maintenance, and stimulus comparison.
Partha Mitra: Developmental learning: How the zebra finch learns its song.

FEBRUARY

Mike Hemann (Lowe Lab): Myc and RNAi: Tails of a lymphomaniac.
Rui-Ming Xu: Structural study of transcriptional silencing.
Zachary Lippman (Martienssen Lab): Transposons, heterochromatin, and the evolution of an epigenome.

MARCH

Ed Ruthazer (Cline Lab): The influence of visual experience on retinal axon development.
Andy Neuwald: Evolutionary clues to DNA polymerase III clamp loader structural mechanisms.
Rotem Karni (Krainer Lab): To splice or not to splice...? The involvement of splicing in transformation and apoptosis.

APRIL

Elena Ezhkova (Tansey Lab): Cracking the histone code with the help of the proteasome.
Niraj Tolia (Joshua-Tor Lab): High-throughput predictive crystallization and its application to malaria invasion processes.

OCTOBER

Masafumi Muratani (Tansey Lab): Lost in translation: Finding ubiquitin in mRNA quality control.
Shouzhen Xia (Tully Lab): NMDA receptors mediate olfactory learning and memory in *Drosophila*.
Prasanth Kannanganattu (Spector Lab): A nuclear retained regulatory RNA transcribed from a protein coding gene.
Gordon Shepherd (Svoboda Lab): Synaptic circuits in a sensory cortex.

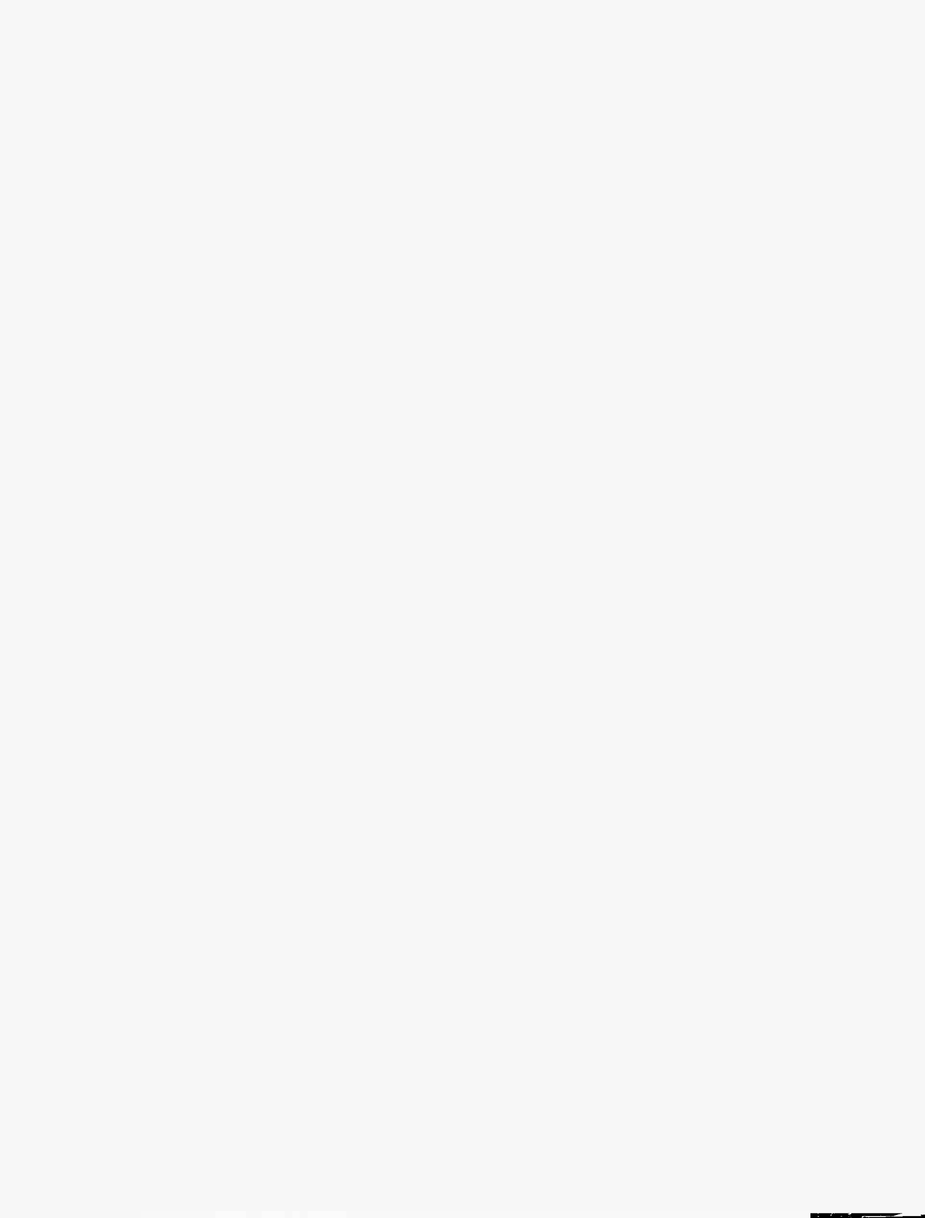
NOVEMBER

Michelle Hastings (Krainer Lab): Wrong site, wrong splice, wrong message: Election of splice sites in two genetic diseases.
Simon Rumpel (Zador Lab): Detecting learning-induced plasticity in the brain: Synaptic and circuit mechanisms of memory formation.
Ji-Joon Song (Joshua-Tor Lab): Finding slicer.

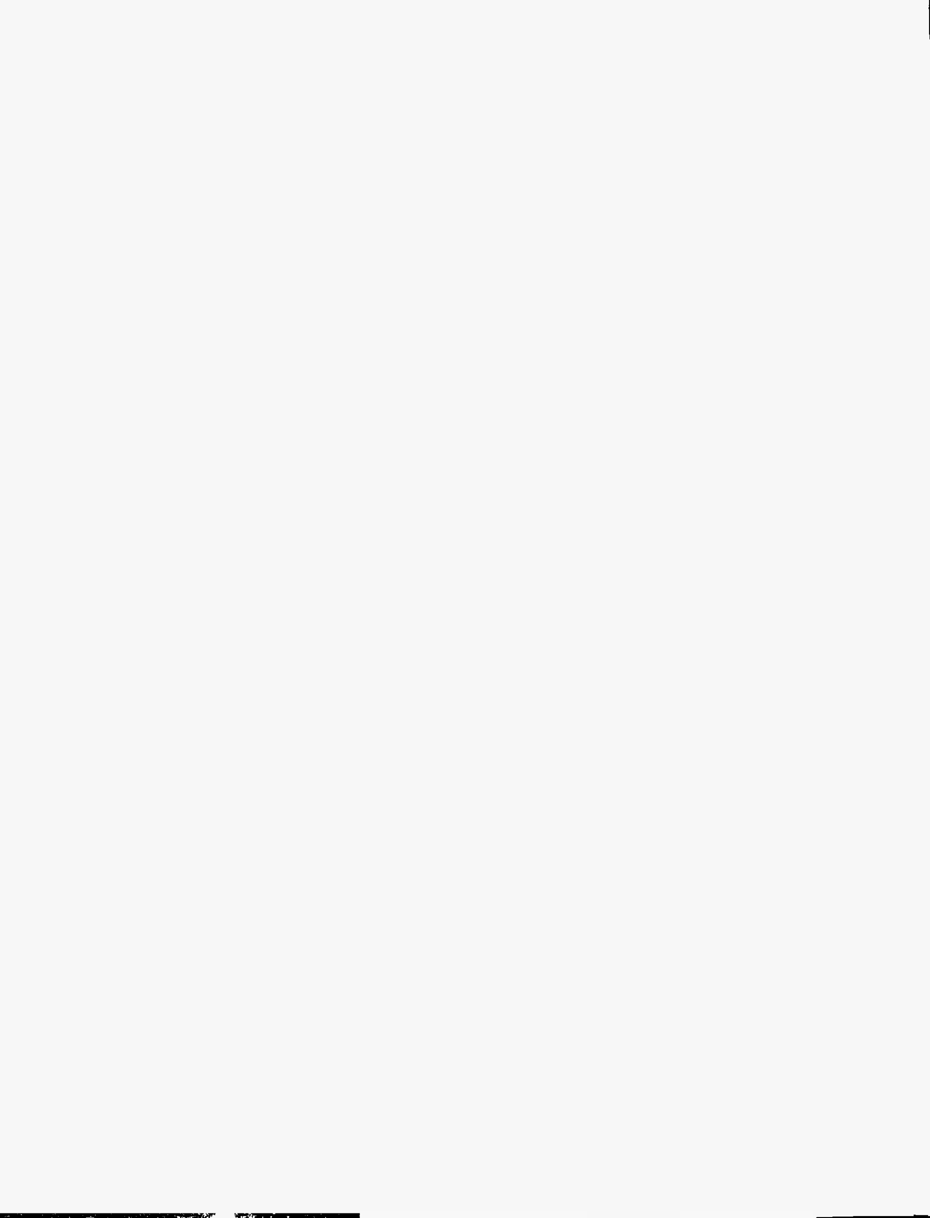
DECEMBER

Jidong Liu (Hannon Lab): Dissecting the mechanism of RNA interference.
Debopriya Das (Zhang Lab): Inferring functional regulatory elements and their cooperativity from microarray data using MARSMotif: Case studies from yeast and mammals.

Lincoln Stein



BANBURY CENTER



BANBURY CENTER EXECUTIVE DIRECTOR'S REPORT

Banbury Center continues to be a thriving setting for specialized, focused meetings on pressing and emerging topics in biomedical research. In 2004, there were 18 meetings at Banbury Center, with 568 invited participants; 80% of the latter came from the United States, representing 38 states. As usual, New York, California, Maryland, and Massachusetts contributed the most. Foreign scientists came to Banbury from 21 countries. The Center continues to be used intensively all year round except during the depths of winter. In addition to the regular program of meetings, the Watson School of Biological Sciences held two week-long *Topics in Biology* courses, and there were five lecture courses in the Laboratory's summer neuroscience program. Two local groups also made use of the Center.

The 2004 Banbury Center program again explored a variety of topics, ranging from discussions on genetic disorders to exploratory meetings on theoretical biology. This year also marked a significant increase in meetings related to neurobiology, a shift that mirrors the Laboratory's expanding programs on brain development and disease. In March, the Center hosted a meeting on the severe neuromuscular disorder: spinal muscular atrophy. The meeting—*Spinal Muscular Atrophy: What Is the Molecular Basis of Neuron Loss?*—organized by Adrian Krainer (Cold Spring Harbor Laboratory), Alex MacKenzie (Children's Hospital of Eastern Ontario), and Kenneth Fischbeck (National Institute of Neurological Disease and Stroke) and reviewed the molecular pathology of this disorder. We continued our examination of Fragile X syndrome with the fifth meeting in a series on that disorder, *New Pharmacological and Neurobiological Approaches to the Treatment of Fragile X*, organized by Will Spooren (F. Hoffman-La Roche) and William Greenough (University of Illinois at Urbana-Champaign). It is remarkable to follow the way in which these meetings have moved from covering basic research to reviewing possible therapies. *T-type Calcium Channels: Their Role in Normal and Pathological CNS Function*, organized by Rodolfo Llinas (New York University Medical Center) and Ed Perez-Reyes (University of Virginia), went from the molecular biology of these channels to their roles in epilepsy, pain, and manic depression, whereas *Communication in Brain Systems*, organized by Terry Sejnowski (The Salk Institute for Biological Studies), dealt much more with theoretical issues, using modeling approaches to understand the structure and engineering of the brain. A fascinating and ambitious meeting, *Origins and Evolution of the Nervous System*, was organized by Ian Meinertzhagen (Dalhousie University, Halifax, Nova Scotia) and Volker Hartenstein (University of California, Los Angeles). Participants reviewed "simple" organisms to see how the morphology, organization, and functioning of their nervous systems change with the differing behavioral requirements of each organism. Although some organisms were familiar to Banbury—*Escherichia coli*, *Caenorhabditis elegans*, and *Drosophila*—others, including sponges, jellyfish, Ascidians, and planarians, made their first appearance at a Banbury Center meeting.

Another parallel to current Laboratory research was our meeting in August on RNAi (RNA interference). One remarkable thing about RNAi is that it provides a tool for the experimental manipulation of gene expression and we are also continuing to discover that it has a key role in cellular function. *RNAi-related Processes in Plants: Chromatin, Development, and Defense*, organized by James Carrington (Oregon State University), Steve Jacobson (University of California, Los Angeles), and Detlef Weigel (Max-Planck Institute for Developmental Biology), reviewed the ways in which plants use RNAi in their everyday life. The timing was just right—participants were able to hear first-hand from Leemor Joshua-Tor and Greg Hannon about their latest studies on Dicer, published a few weeks earlier in the journal *Science*.

Robertson House, the former family residence

Issues of bioterrorism continue to raise great concerns. The Department of Homeland Security funded *Microbial Forensics*, a follow-up meeting to one held in 2003. Organized by Bruce Budowle (Federal Bureau of Investigation Laboratory), Steven Schutzer (UMDNJ–New Jersey Medical School), and James Burans (U.S.

Meier House provides housing accommodations for participants at Banbury Center

Department of Homeland Security), participants discussed issues relating to novel infectious diseases affecting humans, agriculturally important animals, and crop plants. In addition to technical discussions about new methods for the rapid identification of pathogens, there were reviews of gaps in procedures and what the first steps should be to ensure that these gaps are filled. Infectious diseases of a different kind were also explored at the annual meeting sponsored by the Albert B. Sabin Vaccine Institute—*Pandemic Disease Threat: Can We Develop a Global Vaccine Policy?*—which was organized by Lewis A. Miller (Intermedia, Inc.), Dean D. Mason, and Veronica Korn (both from the Albert B. Sabin Vaccine Institute). Vaccination remains the most effective and most cost-effective way to prevent infectious diseases, and yet there is probably less attention currently paid to developing and distributing vaccines than at any previous time.

As in many other institutes around the world, genome-based genetics research is a major Laboratory focus and the major focus of meetings at Banbury. There are great expectations that the intricacies of genetic mutations will be elucidated using genomic tools, and some of these were included in *Breast Cancer Research: A Critical Review for Future Strategies*. Bruce Stillman (Cold Spring Harbor Laboratory) and Joe Sambrook (Peter MacCallum Cancer Institute) organized this small but intense meeting, which had a strong international cast. Understanding what a DNA sequence means continues to be a major theoretical and experimental challenge. *Finding the Functional Elements of the Genome*, organized by Ewan Birney (European Bioinformatics Institute), Aravinda Chakravarti (Johns Hopkins University School of Medicine), Lincoln Stein (Cold Spring Harbor Laboratory), and Richard Young (Whitehead Institute for Biomedical Research), covered topics ranging from comparative genomics to using experimental techniques to find transcriptional regulatory elements. One of the most exciting areas of genetics research is in epigenetics—those changes in genetic information that do not affect the DNA sequence itself yet can be inherited. But there are problems in deciding how to integrate epigenetic data in DNA sequence databases. *Bioinformatic Strategies for the Epigenome* organized by Denise P. Barlow (Center of Molecular Medicine GmbH of the Osterreichische Akademie der Wissenschaften) and Robert Martienssen (Cold Spring Harbor Laboratory) tackled how this might be done.

There is an increasing emphasis on theoretical and mathematical modeling approaches for ordering and understanding biological data. *Integrating Disparate Data to Simulate Lymphocyte Function*, organized by Suzanne Vernon and William C. Reeves (Centers for Disease Control and Prevention), examined whether there is sufficient information about the functions of lymphocytes, and their interactions with other cells, to model their behavior during infection. Talks included such recondite ones as "Application of probabilistic inference and machine learning to lymphocyte function."

Banbury Center has had a continuing interest in promoting genetics education. We held such meetings for science journalists and Congressional staff in conjunction with the Dolan DNA Learning Center and planned meetings on genetics education for nurses. A similar meeting, *Summit Meeting on Genetic Training*, held in 2004, was organized by Bruce Korf (University of Alabama, Birmingham). Participants included representatives from the key professional associations involved with genetics and genetic counseling. They came to review the current state of genetic teaching to physicians and other healthcare professionals, and to discuss what might be done to give genetics training a higher profile in medical education.

The success of the Banbury Center depends on many people. Bea Toliver, Ellie Sidorenko, and Katya Davey make sure that the meetings run properly, and Chris McEvoy and Joe Ellis keep the estate looking beautiful. The audiovisual staff, housekeeping, and the meetings office work closely with us as the Laboratory's meetings programs expand. And, of course, the Center could not work at all without the enthusiasm of organizers and participants.

Jan Witkowski
Executive Director

Spinal Muscular Atrophy: What Is the Molecular Basis of Neuron Loss?

March 7–10

FUNDED BY **Spinal Muscular Atrophy Foundation**

ARRANGED BY **K.H. Fischbeck,** National Institute of Neurological Disorders and Stroke, NIH
A. Krainer, Cold Spring Harbor Laboratory
A. MacKenzie, Children's Hospital of Eastern Ontario

Introduction and Welcome: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
D. Singh, Spinal Muscular Atrophy Foundation, New York
A. MacKenzie, Children's Hospital of Eastern Ontario, Ottawa, Canada

SESSION 1: The Path to SMA Therapeutics: The Big Picture
Chairperson: S.C. Landis, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: NINDS mission

J. Heemskerk, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: The SMA project: Therapeutics development at NINDS.

G. Dreyfuss, K. Fischbeck

SESSION 2: Animal Models of Motor Neuron Disease I
Chairperson: S.C. Landis, National Institutes of Health/ NINDS, Bethesda, Maryland

C.E. Beattie, The Ohio State University, Columbus: Motor neuron development in a zebrafish model of SMA.
T.M. Jessell, Columbia University, New York: Motor neuron ontogeny.
A.H.M. Burghes, Ohio State University, Columbus: SMA mice: When and where SMN corrects of phenotype.
J. Melki, INSERM, Evry, France: Mouse models of SMA: Valuable tools to evaluate pathophysiology and to design therapeutic strategies.

SESSION 3: Animal Models of Motor Neuron Disease II
Chairperson: A.H.M. Burghes, Ohio State University, Columbus

C.J. DiDonato, Children's Memorial Institute for Education & Research, Chicago, Illinois: How to evaluate in vivo testing of drugs in animal models.
G.A. Cox, The Jackson Laboratory, Bar Harbor, Maine: Transgenic rescue of motor neuron disease in the *nmd* mouse model of SMARD1.
A.M. Schaefer, Washington University School of Medicine, St. Louis, Missouri: Motor axon pruning and growth in a mouse model of ALS: In vivo studies.
E. Holzbaur, University of Pennsylvania, Philadelphia: Axonal transport defects in motor neuron degeneration.

D. Singh, G. Fischbach

SESSION 4: SMN Splicing, Function, and Cellular Sublocalization I
Chairperson: A.H.M. Burghes, Ohio State University, Columbus

A. Krainer, Cold Spring Harbor Laboratory: Determinants of exon 7 inclusion in SMN1/SMN2.

SESSION 5: SMN Splicing, Function, and Cellular Sublocalization II
Chairperson: K.H. Fischbeck, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland

G. Dreyfuss, HHMI/University of Pennsylvania School of Medicine, Philadelphia: Functions of SMN.
U. Fischer, University of Wuerzburg, Germany: Analysis of the cellular function of SMN: Implications for spinal muscular atrophy.
G.J. Bassell, Albert Einstein College, Bronx, New York: Active transport of the survival of motor neuron protein in axons and growth cones.
M. Sendtner, Universitat Wuerzburg, Germany: Axonal defects in motor neurons from mouse models of SMA.

SESSION 6: Axonal Pathfinding, Maturation, and Maintenance I
Chairperson: K.H. Fischbeck, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland

L.F. Parada, University of Texas Southwestern Medical Center, Dallas: Lessons to be learned from modeling neurofibromatosis.
S.L. Pfaff, Salk Institute, La Jolla, California: LIM transcription factors and spinal motor neuron development.

SESSION 7: Axonal Pathfinding, Maturation, and Maintenance II
Chairperson: G.D. Fischbach, Columbia University, New York

K. Zinn, California Institute of Technology, Pasadena: Development and maintenance of neuromuscular junctions.
O. Steward, University of California, Irvine: Sorting and intracellular transport of mRNA in neurons.
H. Keshishian, Yale University, New Haven, Connecticut: Synaptic plasticity in model genetic organism.

SESSION 8: Pharmacologic and Cellular Approaches to, and Clinical Trial Design for, SMA Therapy I
Chairperson: G.D. Fischbach, Columbia University, New York

M. Gurney, deCode Genetics, Inc., Woodbridge, Illinois: Drug discovery step by step.
G. Dreyfuss, HHMI/University of Pennsylvania School of Medicine, Philadelphia: SMA therapeutics development.
B.R. Stockwell, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Diagramming disease networks using chemical and biological tools.

SESSION 9: Pharmacologic and Cellular Approaches to, and Clinical Trial Design for, SMA Therapy II
Chairperson: D.C. DeVivo, The Neurological Institute, New York

B. Wirth, Institute of Human Genetics, Bonn, Germany: Search for drugs that increase the expression SMN2.
C.T. Sumner, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: Histone deacetylase inhibitors as treatment for spinal muscular atrophy.
A. MacKenzie, Children's Hospital of Eastern Ontario, Ottawa, Canada: Apoptosis-based modulation of SMA.
D. Kerr, Johns Hopkins Hospital, Baltimore, Maryland: Embryonic stem-cell-derived motoneurons from SMA mice.
T. Crawford, Johns Hopkins Hospital, Baltimore, Maryland: Natural history of human SMA and potential surrogate measures necessary to powering clinical trials.
K.J. Swoboda, University of Utah School of Medicine, Salt Lake City: Phenotype/genotype correlates: SMN2 copy number, SMN protein measurement, data, and the role of modifying factors.

SESSION 10: Where Do We Go From Here? Setting Priorities for Finding Therapies for SMA
Discussion Leader: K.H. Fischbeck, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland

Finding the Functional Elements of the Genome

March 21–24

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **E. Birney,** European Bioinformatics Institute
A. Chakravarti, Johns Hopkins University School of Medicine
L. Stein, Cold Spring Harbor Laboratory
R. Young, Whitehead Institute for Biomedical Research

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Genome Sequence and Protein-coding Genes
Chairperson: E. Birney, European Bioinformatics Institute, Cambridge, United Kingdom

E. Green, National Human Genome Research Institute, NIH, Bethesda, Maryland: Identification of highly conserved genomic elements by multispecies comparative sequencing.
M. Zody and M. Clamp, The Broad Institute, Cambridge, Massachusetts: Feasibility of annotating the human genome through light sequencing of multiple mammals.
B.A. Cohen, University of Washington School of Medicine, St. Louis, Missouri: Comparative genomics in yeast.
D. Kulp, University of Massachusetts, Amherst: A general model of experimental transcriptional evidence.
C.P. Ponting, University of Oxford, United Kingdom: Variations in evolutionary rates of mammalian genes.

SESSION 2: Experimental Techniques to Discover Function
Chairperson: A. Chakravarti, Johns Hopkins University School of Medicine, Baltimore, Maryland

D. Baillie, Simon Fraser University, Burnaby British Columbia, Canada: Using SAGE to identify functional elements in the *C. elegans* genome.
J. Schimenti, The Jackson Laboratory, Bar Harbor, Maine: Relative merits of various in vivo functional genomics strategies in mice.
M. Vidal, Dana-Farber Cancer Institute, Boston, Massachusetts: Networks in biology.
R.A. Young, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: A draft transcriptional regulatory code for yeast.
J. Hein, University of Oxford, United Kingdom: Two ideas: Rooting by irreversibility and disentangling overlapping selective constraints.

J. Hein, R. Wilson

SESSION 3: Genome-wide Conservation Analysis
Chairperson: E. Green, National Human Genome Research Institute, NIH, Bethesda, Maryland

M. Dermitzakis, University of Geneva Medical School, Switzerland: Divergence, polymorphism, and evolutionary characteristics of conserved nongenic sequences.

L. Pachter, University of California, Berkeley: Phylogenetic methods in comparative genomics and applications to functional element identification.

G. Bejerano, University of California, Santa Cruz: Large-scale clustering and analysis of human noncoding DNA.

SESSION 4: Specific Nongenic Features I
Chairperson: L.D. Stein, Cold Spring Harbor Laboratory

S. Griffiths-Jones, The Wellcome Trust Sanger Institute, Cambridge, United Kingdom: Progress toward a one-stop noncoding RNA annotation shop.

E. Fraenkel, Whitehead Institute, Cambridge, Massachusetts: Combining diverse data sources to identify transcription-factor-binding sites.

G.D. Stormo, Washington University Medical School, St. Louis, Missouri: Finding regulatory motifs using coregulated genes from multiple species.

E. Birney, European Bioinformatics Institute, Cambridge, United Kingdom: *Cis*-regulatory discovery in vertebrates.

E. Segal, Stanford University, California: Genome-wide discovery of *cis*-regulatory modules using sequences, expression, and location data.

S. Jones, Genome Sciences Centre, Vancouver, British Columbia, Canada: Integrated approaches to regulatory element detection using the Sockeye platform.

A. Chakravarti, Johns Hopkins University School of Medicine, Baltimore, Maryland: Coding and noncoding mutations in human complex disease.

L.D. Stein, Cold Spring Harbor Laboratory: An ontology of functional elements.

Summary

E. Birney, European Bioinformatics Institute, Cambridge, United Kingdom
A. Chakravarti, Johns Hopkins University School of Medicine, Baltimore, Maryland
L.D. Stein, Cold Spring Harbor Laboratory
R.A. Young, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

J. Kent, D. Kulp

Neuronal and Behavioral Effects of Ras/MAPK Signaling

April 4–7

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **A. Silva,** University of California, Los Angeles
J.D. Sweatt, Baylor College of Medicine
L. Van Aelst, Cold Spring Harbor Laboratory
J.J. Zhu, University of Virginia School of Medicine

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SPECIAL SESSION
Chairperson: L. Van Aelst, Cold Spring Harbor Laboratory: Introduction

S. Tonegawa, Massachusetts Institute of Technology, Cambridge: Translational control by MAPK signaling in long-term synaptic plasticity and memory.

E.R. Kandel, HHMI/Columbia University, New York: On molecular mechanisms for synaptic growth and the perpetuation of long-term synaptic plasticity.

SESSION 1: Learning and Memory
Chairperson: A. Silva, University of California, Los Angeles: Introduction

J.D. Sweatt, Baylor College of Medicine, Houston, Texas: Regulation and targets of ERK in the hippocampus.

S.A. Siegelbaum, Columbia University College of Physicians & Surgeons, New York: p38 MAPK/12-lipoxygenase signaling in mGluR LTD.

K. Rosenblum, Haifa University, Israel: Role of MAPK in memory and LTP consolidation.

D.R. Storm, University of Washington School of Medicine, Seattle: A new mechanism for sensitization of Erk MAP kinase during memory formation.

R. Nicoll, D. Bredt, E. Kandel

E. Kandel, J.D. Watson

SESSION 2: Learning and Memory
Chairperson: R. Malinow, Cold Spring Harbor Laboratory: Introduction

R. Brambilla, DIBIT-HSR, Milano, Italy: Ras/MAPK signalling in stratum-dependent behavioral plasticity.

Y. Elgersma, Erasmus MC, Rotterdam, The Netherlands: Role of presynaptic Ras signaling in learning and memory.

P. Stork, Vollum Institute, Oregon Health Sciences University, Portland: Rap1 activation of ERKs in neuronal cell types.

SESSION 3: Cognitive Disorders and Pathology
Chairperson: J.J. Zhu, University of Virginia School of Medicine, Charlottesville: Introduction

A. Silva, University of California, Los Angeles: Role of neurofibromin in the regulation of synaptic inhibition, plasticity, and learning and memory: Implications to the treatment of NF1.

Y. Zhong, Cold Spring Harbor Laboratory: Ras-Giap (NF1)-medicated signaling in learning vs. memory.

L. Mucke, University of California, San Francisco: Behavioral deficits in transgenic models of Alzheimer's disease: Are they due to alterations in MAPK/ERK-related pathways?

S. Dudek, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Somatic vs. synaptic ERK activation: Roles of action potentials, frequency, and mode of calcium entry.

E. Thiels, University of Pittsburgh, Pennsylvania: MAPK in hippocampal synaptic plasticity: How specific of a signal is it?

SESSION 4: Synaptic and Morphological Plasticity
Chairperson: M.H. Sheng, HHMI/Massachusetts of Technology, Cambridge: Introduction

D.S. Bredt, University of California, San Francisco: Stargazin: An AMPA receptor subunit.

G. Thomas, Johns Hopkins School of Medicine, Baltimore, Maryland: Binding and phosphorylation of PDZ domain proteins by RSK2 regulate synaptic transmission.

H. Cline, Cold Spring Harbor Laboratory: Multiple mechanisms of activity-dependent circuit development in vivo.

R.A. Nicoll, Univesity of California, San Francisco: Stargazin/TARPs: Role in AMPAR trafficking and plasticity in the hippocampus.

SESSION 5: Receptors and Channels
Chairperson: J.D. Sweatt, Baylor College of Medicine, Houston, Texas: Introduction

J.W. Hell, University of Iowa, Iowa City: Regulation of postsynaptic functions by calmodulin, CaMKII, and α-actinin.

B. Lu, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: Molecular mechanisms underlying acute and long-term synaptic modulation by neurotrophins.

D. Johnston, Baylor College of Medicine, Houston, Texas: MAPK regulation of dendritic K^{+} channels in hippocampal neurons.

L. Mei, University of Alabama, Birmingham: Prenylation as a novel mechanism in synapse formation and synaptic plasticity.

S. Tonegawa, D. Johnston, K. Rosenblum, and D. Sweatt

Microbial Forensics

April 18–21

FUNDED BY **U.S. Department of Homeland Security (through a grant to the UMDNJ–New Jersey Medical School**

ARRANGED BY **B. Budowle,** FBI Laboratory
J. Burans, U.S. Department of Homeland Security
S.E. Schutzer, UMDNJ–New Jersey Medical School

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
B. Budowle, FBI Laboratory, Quantico, Virginia
S.E. Schutzer, UMDNJ–New Jersey Medical School, Newark

SESSION 1: Scenarios Where Microbial Forensics May Be Applied
Chairperson: S.E. Schutzer, UMDNJ–New Jersey Medical School, Newark

J.P. Burans, U.S. Department of Homeland Security, Frederick, Maryland: Mission, operation, and structure of the National Bioforensic Analysis Center. Needs of the BFAC.
B. Budowle, FBI Laboratory, Quantico, Virginia: Points to consider for microbial forensics.
R.S. Murch, Institute for Defense Analysis, Alexandria, Virginia: Scenario I: Description and interactive participation with audience expertise.
R.S. Murch, Institute for Defense Analysis, Alexandria, Virginia: Scenario II: Description and interactive participant with audience expertise.

SESSION 2: Collection, Handling, and Analysis of Evidentiary Samples
Chairperson: B.J. Luft, Stony Brook University, New York

D. Beecher, FBI Laboratory, Quantico, Virginia: Current methods and FBI protocols for sample collection and handling: Bulk material and traces of bacteria, viruses, and toxins.
R. Okinaka, Los Alamos National Laboratory, New Mexico: Merging SNP discovery and rapidly evolving markers toward forensic signatures.

B. Budowle, A. Walsh, J. Dunn, S. Schutzer

R. Winegar, Midwest Research Institute, Palm Bay, Florida: Extraction and recovery of minute quantities of nucleic acids from dilute samples and complex matrices.

J. Ravel, Microbial Genomics, Rockville, Maryland: High-throughput sequencing and comparative genomic potentials for microbial forensics.

P.J. Jackson, Los Alamos National Laboratory, New Mexico: Pathogen genotyping.

SESSION 3: Scientific Approaches (II) and Interpretation
Chairpersons: P.J. Jackson, Los Alamos National Laboratory, New Mexico; **D.L. Rock,** USDA Agricultural Research Service, Greenport, New York

B. Budowle, FBI Laboratory, Quantico, Virginia: Preservation of traditional forensic material: Finding trace evidence to link a suspect to biocrime/bioterrorism.

S. Velsko, Lawrence Livermore National Laboratory, California: Instrumental analysis and interpretation.

R.B. Harris, Commonwealth Biotechnologies, Inc., Richmond, Virginia: Analysis, confirmation, and identification of bio-agent toxins: A tiered mass spectrometry approach.

R. Chakraborty, University of Cincinnati, Ohio: Issues of diversity and variation and impact on interpretation.

D.J. Ecker, IBIS Therapeutics, ISIS Pharmaceuticals, Carlsbad, California: Combinatorial methodologies as applied to microbial forensics.

SESSION 4: Animal Pathogens; Host Response
Chairperson: D.R. Franz, Midwest Research Institute, Frederick, Maryland

D.L. Rock, USDA Agricultural Research Service, Greenport, New York: Comparative and functional genomics: Application for human and animal microbial forensics.

B.J. Luft, SUNY, Stony Brook, New York and M.S. Ascher, Lawrence Livermore National Laboratory, California: Antibiotic bioavailability in the host as a potential bioforensic marker: Half-lives and tissue deposition.

S.E. Schutzer, UMDNJ–New Jersey Medical School, Newark: Temporal host responses marking specific pathogen exposure.

SESSION 5: Lessons Not Yet Learned: Critical Gaps in Microbial Forensics
Chairpersons: B. Budowle, FBI Laboratory, Quantico, Virginia; **R. Breeze,** Washington, D.C.

S.A. Morse, Centers for Disease Control and Prevention, Atlanta, Georgia: Role of public health system.

B. Budowle and J. Bannan, FBI Laboratory, Quantico, Virginia: Role of the FBI in coordinating investigation of biocrime and bioterrorism with local law enforcement.

J.W. Ezzell, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland; L.W. J. Baillie, Noval Medical Research Center, Silver Spring, Maryland; D. Beecher, FBI Laboratory, Quantico, Virginia; and T. Slezak, Lawrence Livermore National Laboratory, California: Experiences from anthrax and ricin attacks.

SESSION 6: The Judicial System
Chairperson: L.W.J. Baillie, Naval Medical Research Center, Silver Spring, Maryland

R.P. Harmon, Alameda County District Attorney's Office, Oakland, California: Legal issues: Examples from previous court cases.

SESSION 7: Critical Issues and How to Proceed to Address the Gaps before the Next Attack
Chairpersons: B. Budowle, FBI Laboratory, Quantico, Virginia; **S.E. Schutzer,** UMDNJ–New Jersey Medical School, Newark

R. Breeze, Washington, D.C.: Agricultural biocrimes are fundamentally different from attacks on people.

Items for the BFAC

Items That Should be Discussed at all Levels of Government

Research Needed to Fill Gaps in Bioforensics

M. Ascher, J. Ravel, R. Chakraborty

New Pharmacological and Neurobiological Approaches to the Treatment of Fragile X

April 25–28

FUNDED BY **NIH–National Institute of Mental Health (through a grant to University of Illinois)**

ARRANGED BY **W.T. Greenough,** University of Illinois, Urbana-Champaign
W. Spooren, F. Hoffmann-La Roche

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Fragile X: The Clinical Perspective
Chairperson: E. Berry-Kravis, Rush Children's Hospital, Chicago, Illinois

K. Clapp, FRAXA Research Foundation, Newburyport, Massachusetts: Fragile X: A patient perspective.
D. Bailey, University of North Carolina, Chapel Hill: The Fragile-X syndrome: Phenotype overview and patterns of medication use.
R.J. Hagerman, University of California Davis Health System, Sacramento: Psychopharmacological interventions in Fragile X.
S.T. Warren, Emory University School of Medicine, Atlanta, Georgia: Molecular mechanisms of Fragile-X phenotype from a molecular perspective.

SESSION 2: FMR-1 Mouse Models
Chairperson: W.T. Greenough, University of Illinois, Urbana

T. Steckler, Johnson & Johnson Pharmaceutical, Beerse, Belgium: Mutant mouse: Where hopes meet reality.
R.E. Paylor, Baylor College of Medicine, Houston, Texas: Behavior of Fmr/KO mice: Role of genetic background and therapeutic interventions.
B.A. Oostra, Erasmus Universiteit Rotterdam, The Netherlands: Enhanced LTD at enlarged Purkinje cell spines in the cerebellum causes motor learning deficits in Fragile-X syndrome.
M. Toth, Cornell University Medical College, New York: Hyperactivity of Fragile-X mice to sensory stimuli: Network hyperexcitability and alterations in FMRP-target expression.

D. Nelson, B. Oostra, E. Klann, P. Jin, S. Warren

SESSION 3: FMR1 and the Neurobiology of Fragile X
Chairperson: D.L. Nelson, Baylor College of Medicine, Houston, Texas

R.K.S. Wong, SUNY-Health Science Center, Brooklyn, New York: Metabotropic glutamate receptors and epileptogenesis in Fragile-X syndrome.
R.P. Bauchwitz, St. Luke's–Roosevelt Institute of Health Sciences, Columbia University, New York: What elevated audiogenic seizure susceptibility in Fragile-X mice reveals about the etiology of Fragile-X syndrome.
P.W. Vanderklish, Scripps Research Institute, La Jolla, California: Regulatory interactions between synaptic structure and local translation.
E. Klann, Baylor College of Medicine, Houston, Texas: Regulation of translation signaling pathways during mGlur-LTD in Fmr1 knockout mice.
J. Julius Zhu, University of Virginia School of Medicine, Charlottesville: Ras signaling of excitatory synapses of FMR1 knockout mice.
M.F. Bear, HHMI/Massachusetts Institute of Technology, Cambridge: The mGluR theory of Fragile X in mental retardation.

SESSION 4: Emerging Drugable Targets: mGlu Receptors
Chairpersons: W. Spooren, F. Hoffmann-La Roche, Basel, Switzerland; **G. Bilbe,** Novartis Institutes for BioMedical Research, Basel, Switzerland

M.R. Tranfaglia, FRAXA Research Foundation, Newburyport, Massachusetts: Clinical/psychiatric presentation of Fragile X.
G. Bilbe, Novartis Pharma AG, Basel, Switzerland: A drug discovery perspective: Translation from bench to bedside.
V. Mutel, Addex Pharmaceuticals SA, Geneva, Switzerland: Drugability of targets.
M.P. Johnson, Eli Lilly and Company, Indianapolis, Indiana: Advances in glutamate receptor pharmacology: Targets galore.
F. Gasparini, Novartis Pharma AG, Basel, Switzerland: Allosteric modulation for the mGlu receptors: Antagonists, positive modulators, neutral ligands.
T. Steckler, Johnson & Johnson Pharmaceutical, Beerse, Belgium: CRF, antagonists as anxiolytic and antidepressant drugs.
W. Spooren, F. Hoffmann-La Roche, Basel, Switzerland: Neurokinin 3 (NK3) receptors: A new target for the treatment of psychosis?
G.R. Dawson, Merck Sharp & Dohme Research Laboratories, Essex, United Kingdom: Role of GABA-A receptor subtypes in anxiety and cognition.
B. Bettler, Universitat Basel, Switzerland: GABA-B receptors: Drug targets for Fragile-X disorders?
C. Dobkin, New York State Institute for Basic Research, Staten Island: Seizure susceptibility of the Fragile-X mouse and alterations of the GABAergic system.

SESSION 5: Developing Drugs for Fragile X
Chairperson: D. Bailey, University of North Carolina, Chapel Hill

T.A. Jongens, University of Pennsylvania School of Medicine, Philadelphia: Effect of pharmacological interventions (MPEP) on phenotype in the *Drosophila* Fragile-X mutant MPEP: Results in *Drosophila*.
P. Jin, Emory University, Atlanta, Georgia: Potential roles of microRNA and MPEP in Fragile-X syndrome.
H.G.M. Westenberg, University Medical Center Utrecht, The Netherlands: On the role of dopamine in anxiety disorders: Recent findings OCD and social anxiety disorder.
E. Berry-Kravis, Rush Children's Hospital, Chicago, Illinois: Clinical trial of Ampakine CX516 in Fragile-X syndrome.

SESSION 6: Strategies and Goals for Developing Therapies
Moderators: M.R. Tranfaglia, FRAXA Research Foundation, Newburyport, Massachusetts; **W. Spooren,** F. Hoffmann-La Roche, Basel, Switzerland

J.-L. Claverie, B. Oostra

Breast Cancer Research: A Critical Review for Future Strategies

May 9–12

FUNDED BY **Den Haag Foundation**

ARRANGED BY **J. Sambrook,** Peter MacCallum Cancer Institute
B. Stillman, Cold Spring Harbor Laboratory

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
B. Stillman, Cold Spring Harbor Laboratory
J. Sambrook, Peter MacCallum Cancer Institute, Melbourne, Australia

SESSION 1: Epidemiology and Risk Assessment
Chairperson: B.J. Ponder, Cancer Research United Kingdom, Cambridge

D. Easton, Cancer Research United Kingdom, Cambridge: Risk models for breast cancer: What they tell us and what we still need to know.

M. Dowsett, Royal Marsden Hospital, London, United Kingdom: Fitting steroid analyses into risk algorithms for breast cancer: Opportunities and challenges.

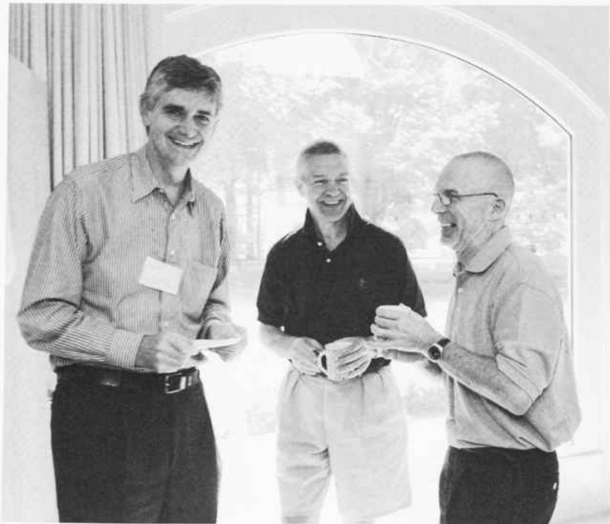
Discussion Moderator: B.J. Ponder, Cancer Research United Kingdom, Cambridge

SESSION 2: Stem Cells and Development
Chairperson: L.A. Chodosh, University of Pennsylvania School of Medicine, Philadelphia

T.D. Tlsty, University of California, San Francisco: Early genetic and epigenetic events in breast cancer.

E.M. Rosen, Georgetown University, Washington, D.C.: BRCA1-endocrine interactions: Implications for breast cancer control and therapy EMR.

Discussion Moderator: L.A. Chodosh, University of Pennsylvania School of Medicine, Philadelphia



B. Stillman, J.A. Witkowski, J. Sambrook

SESSION 3: Genetics
Chairperson: G. Chenevix-Trench, The Queensland Institute of Medical Research, Herston, Australia

K. Offit, Memorial Sloan-Kettering Cancer Center, New York: Linkage disequilibrium mapping: A tool for breast cancer susceptibility gene rediscovery?
B.J. Ponder, Cancer Research United Kingdom, Cambridge: Low-penetrance genes and implications for prevention.
M.-C. King, University of Washington School of Medicine, Seattle: An approach for finding more genes for inherited breast cancer.

Discussion Moderator: G. Chenevix-Trench, The Queensland Institute of Medical Research, Herston, Australia

SESSION 4: Decision Making
Chairperson: C. Scott, Cold Spring Harbor Laboratory

K. Armstrong, University of Pennsylvania, Philadelphia: Moving into clinical practice: Technology assessment/adoption and diffusion.

Discussion Moderator: C. Scott, Cold Spring Harbor Laboratory

SESSION 5: Genomic Changes and Targets I
Chairperson: T. Tlsty, University of California, San Francisco

A. Zetterberg, Karolinska Institutet, Stockholm, Sweden: Analysis of deletions and amplications in breast cancer with quantitative multigene FISH.
M. Wigler, Cold Spring Harbor Laboratory: Combinatorial approaches to identification of targets.
J. Hicks, Cold Spring Harbor Laboratory: ROMA analysis of group cancer cells.
J.W. Gray, Lawrence Berkeley National Laboratory, Berkeley, California: Genomic events in breast cancer: Diagnostic and therapeutic opportunities.
A.-L. Borresen-Dale, The Norwegian Radium Hospital, Oslo, Norway: Challenges of combining large-scale genomic data of tumors with patients' genotype and clinical outcome.
L. Norton, Memorial Sloan-Kettering Cancer Center, New York: Strategies for improving breast cancer therapy by the use of mathematical models of tumor growth kinetics and the integration of molecular profiling in clinical trials.

SESSION 6: Genomic Changes and Targets II
Chairperson: B. Stillman, Cold Spring Harbor Laboratory

D. Sgroi, Massachusetts General Hospital, Charlestown: Expression profiling of human breast cancer with capture microdissection.

Discussion Moderator: E.M. Rosen, Georgetown University, Washington, D.C.

SESSION 7: Animal Models
Chairperson: S. Lowe, Cold Spring Harbor Laboratory

J. Green, National Cancer Institute, Bethesda, Maryland: Genomic approaches to understanding mammary cancer evolution using mouse models.
L.A. Chodosh, University of Pennsylvania School of Medicine, Philadelphia: Genetically engineered mouse models for breast cancer susceptibility. Oncogene reversibility and progression.

Discussion Moderator: S. Lowe, Cold Spring Harbor Laboratory

SESSION 8: Targets and Trials
Chairperson: M. Dowsett, Royal Marsden Hospital, London, United Kingdom

M. Pike, University of California School of Medicine, Los Angeles: Experience with a breast cancer chemoprevention regimen for premenopausal women based on blocking ovarian function with a GnRH agonist.
C. Kent Osborne, Baylor College of Medicine, Houston, Texas: Hormone therapy of breast cancer: How ER and growth factor receptor pathways collaborate to cause treatment failure and how this cross-talk can be overcome to restore effective treatment.

Discussion Moderator: M. Dowsett, Royal Marsden Hospital, London, United Kingdom

SESSION 9: Infrastructure for Breast Cancer Research
Chairperson: J. Sambrook, Peter MacCallum Cancer Research Institute, East Melbourne, Australia

SESSION 10: Summary: Goals and Strategies
Discussion Moderators: B. Stillman, Cold Spring Harbor Laboratory; **J. Sambrook,** Peter MacCallum Cancer Research Institute, East Melbourne, Australia

Communication in Brain Systems

May 16–19

FUNDED BY **The Swartz Foundation and The Alfred P. Sloan Foundation**

ARRANGED BY **T. Sejnowski,** The Salk Institute for Biological Studies

Introductions: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California

SESSION 1: Constraints on Communication
Chairperson: T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California

D. Chklovskii, Cold Spring Harbor Laboratory: Brain architecture maximizes neuronal connectivity while minimizing time delays.
E. Halgren, Massachusetts General Hospital, Charlestown: Intracolumnar vs. intercolumnar communication in the human neocortex.
O. Bertrand, INSERM, Lyon, France: Functional modulations of local and long-distance β/γ oscillatory synchronization: Evidence from direct intracranial recordings in humans.
S. Makeig, University of California, San Diego: Multiscale evidence of multiscale brain communication.

H. Cohen, G. Chin, G. Tanoni

R. Knight, P. Fries, O. Bertrand

SESSION 2: Regulation of Communication
Chairperson: S. Makeig, University of California, San Diego

P. Fries, University of Nijmegen, The Netherlands: Neuronal coherence in man and monkey.
E. Buffalo, National Institutes of Health/NIMH, Bethesda, Maryland: Layer-specific attentional modulation of neuronal synchrony.
T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California: Inhibitory mechanisms for attentional gain control.
G. Rainer, Max-Planck Institute for Biological Cybernetics, Tübingen, Germany: Phase locking of single neuron activity to theta oscillations during working memory in monkey extrastriate visual cortex.
M. Kahana, Brandeis University, Waltham, Massachusetts: From oscillations in the immature and mature human cortex.
K.K. Kaila, University of Helsinki, Finland: Infraslow oscillations in the immature and mature human cortex.

SESSION 3: Computation and Communication
Chairperson: J.M. Allman, California Institute of Technology, Pasadena

E. Salinas, Wake Forest University of Medicine, Winston-Salem, North Carolina: Gain modulation as a mechanism for the selection of functional circuits.
R. Rao, University of Washington, Seattle: Probabilistic models of cortical computation and communication.
J.H. Reynolds, The Salk Institute for Biological Studies, La Jolla, California: Surface-based attention determines dominance in binocular rivalry.
X.-J. Wang, Brandeis University, Waltham, Massachusetts: Cortical circuits of working memory and decision-making.

SESSION 4: Communication Infrastructure
Chairperson: D. Chklovskii, Cold Spring Harbor Laboratory

R. Sarpeshkar, Massachusetts Institute of Technology, Cambridge: Hybrid computation with spikes.
P. Mitra, Cold Spring Harbor Laboratory: Metabolic cost of readiness.
G. Tononi, University of Wisconsin, Madison: Traveling waves and cortical connectivity.
R. da Silveira, Harvard University, Cambridge, Massachusetts: Short paths and signal propagation in a model cortex.
D. Ballard, University of Rochester, New York: Prospects for synchronous communication in the cortex.

SESSION 5: Command and Control of Communication

R.T. Knight, University of California, Berkeley: Prefrontal modulation of sensory processing.
Z.F. Mainen, Cold Spring Harbor Laboratory: Prefrontal and olfactory cortical circuits engaged during odor discrimination in the rat.
J.M. Allman, California Institute of Technology, Pasadena: The fronto-insular cortex and the evolution of social cognition.

T-type Calcium Channels: Their Role in Normal and Pathological CNS Function

May 23–25

FUNDED BY **Cold Spring Harbor Laboratory**

ARRANGED BY **R. Llinas,** New York University Medical Center
E. Perez-Reyes, University of Virginia

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Channel Properties
Chairperson: J.R. Huguenard, Stanford University School of Medicine, California

R. Llinas, New York University Medical Center, New York: Calcium T-channel properties and intrinsic neuronal activity.
E. Perez-Reyes, University of Virginia, Charlottesville: Molecular biology of the T-type calcium channel family.
P. Lory, IGH-CNRS UPR, Montpelier, France: New insights into the use of recombinant T-channels to probe neuronal excitability.
Y. Yarom, Hebrew University, Jerusalem, Israel: The density of T-type calcium channels shapes the electrical behavior of the neuron.
T.P. Snutch, University of British Columbia, Vancouver, Canada: Development of novel small molecule T-type calcium channel blockers.

SESSION 2: Rhythms
Chairperson: H.-S. Shin, Korea Institute of Science and Technology, Seoul

D.A. McCormick, Yale University School of Medicine, New Haven, Connecticut: Control of rhythmogenesis in thalamic neurons and networks.
J.R. Huguenard, Stanford University School of Medicine, California: T-channel diversity in thalamic circuits: Functional consequences for rhythm generation.
V. Crunelli, Cardiff University, United Kingdom: Thalamic T-type calcium channels in EEG slow waves.
D. Contreras, University of Pennsylvania, Philadelphia: Thalamic bursting and thalamic quiescence during electrographic seizures.

P. Rhodes, B. Hu, R. Llinas

SESSION 3: T-channel Role in Normal Function
Chairperson: D.A. McCormick, Yale University School of Medicine, New Haven, Connecticut

B. Hu, University of Calgary, Alberta, Canada: T-current and posterior sensory cueing network.

H.-S. Shin, Korea Institute of Science and Technology, Seoul: T-type calcium channels in thalamic sensory gating and regulation of novelty-seeking behavior.

G.A. Marini, Centro di Ricerca Sperimentale, Milano, Italy: Low-frequency (7–12 Hz) neuronal oscillations during brain-activated states of behaving rats.

N. Leresche, Neurobiologie Cellulaire, Paris, France: Paradoxical potentiation of neuronal T-type Ca^{2+} current by ATP at resting membrane potential.

T. Bal, Institut de Neurobiologie Alfred Fessard, Gif-sur-Yvette, France: Background synaptic noise, T current, and signal transfer in thalamocortical cells.

SESSION 4: T Channels in Pathological Conditions
Chairperson: V. Crunelli, Cardiff University, United Kingdom

J.L. Noebels, Baylor College of Medicine, Houston, Texas: Thalamic T-type currents in mutant mouse models of epilepsy.

J. Georgia McGivern, Amgen Inc., Thousand Oaks, California: The potential role of T-type calcium channels in neuropathic pain.

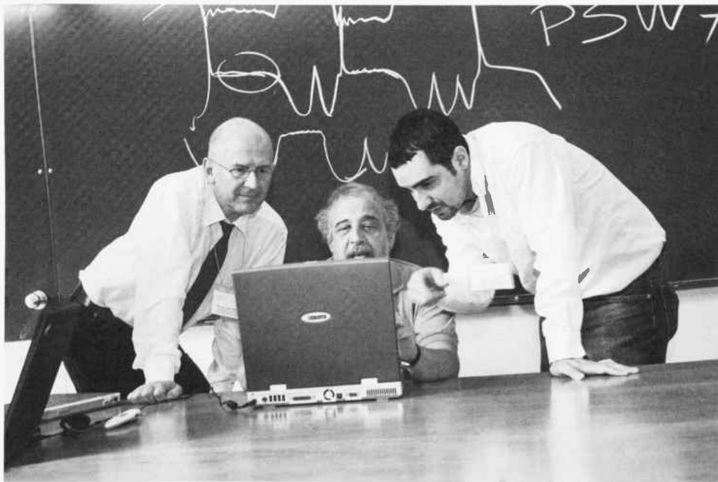
X. Xie, SRI International, Menelo Park, California: Mechanism of lamotrigine actions: Implications in bipolar disorder.

S. Todorovic, University of Virginia, Charlottesville: Volatile anesthetics disrupt signaling in the thalamus mediated by a slowly inactivating T-type calcium channel.

D. Jeanmonod, University Hospital Zurich, Switzerland: Low-threshold calcium spike bursts and the human thalamocortical dysrhythmia.

Goals and Strategies
Moderators: R. Llinas, New York University Medical Center; **E. Perez-Reyes,** University of Virginia

Closing Remarks: J.D. Watson, Cold Spring Harbor Laboratory



D. Jeanmonod, V. Crunelli, R. Ramirez

RNAi-related Processes in Plants: Chromatin, Development, and Defense

August 15–18

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J.C. Carrington,** Oregon State University
S. Jacobsen, University of California, Los Angeles
D. Weigel, Max-Planck Institute for Developmental Biology

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
J.C. Carrington, Oregon State University, Corvallis

SESSION 1: Mechanisms, Components, and Intersections
Chairperson: D. Weigel, Max-Planck Institute for Developmental Biology, Tübingen, Germany

D.C. Baulcombe, The Sainsbury Laboratory, Norwich, United Kingdom: Systemic signaling and epigenetic mechanisms in RNA silencing.

H. Vaucheret, Laboratoire de Biologie Cellulaire, Versailles, France: Interconnections between miRNA and siRNA pathways in plants.

J.C. Carrington, Oregon State University, Corvallis: Diversification, intersection, and evolution of small RNA pathways.

O.C. Voinnet, Institute de Biologie Moleculaire des Plantes du CNRS, Strasbourg, France: In planta imaging of miRNA transcription and endonucleolytic cleavage activity.

G. Hannon, Cold Spring Harbor Laboratory: RNAi: Mechanism and application.

L. Joshua-Tor, Cold Spring Harbor Laboratory: Slicer–Revealed.

D.J. Patel, Memorial Sloan-Kettering Cancer Center, New York: Protein-RNA recognition events in RNA interference.

SESSION 2: RNAi, Chromatin, and Transcriptional Silencing
Chairperson: B. Bartel, Rice University, Houston, Texas

S. Jacobsen, University of California, Los Angeles: RNA-directed chromatin modifications in *Arabidopsis*.

S. Henikoff, Fred Hutchinson Cancer Research Center, Seattle, Washington: DNA methylation profiling identifies targets of epigenetic regulators in *Arabidopsis*.

M. Matzke, Austrian Academy of Sciences, Vienna: Genetic analysis of RNA-mediated transcriptional gene silencing.

J. Bender, Johns Hopkins University, Baltimore, Maryland: RNA-directed DNA methylation of the endogenous PAI genes in *Arabidopsis*.

C.S. Pikaard, Washington University, St. Louis, Missouri: Ribosomal RNA gene silencing.

P.M. Waterhouse, CSIRO Plant Industry, Canberra, Australia: siRNA-mediated methylation in plants.

M. Timmermans, J. Fletcher, K. Barton

SESSION 3: RNAi, Chromatin, and Development
Chairperson: S. Jacobsen, University of California, Los Angeles

V.L. Chandler, University of Arizona, Tucson: Role of tandem repeats in paramutation at the *b1* locus in maize.

R. Martienssen, Cold Spring Harbor Laboratory: Heterochromatin, RNAi, and epigenetic gene control.

C. Dean, J. Innes Centre, Norwich, United Kingdom: Do small RNAs play a role in *FLC* regulation?

R. Amasino, University of Wisconsin, Madison: Epigenetic regulation of flowering via vernalization.

X. Chen, Waksman Institute, Rutgers University, Piscataway, New Jersey: siRNAs in the regulation of natural flowering behavior in *Arabidopsis*.

J.-K. Zhu, University of California, Riverside: Small RNAs, transcriptional gene silencing, and their role in abiotic stress responses.

SESSION 4: microRNAs and Developmental Mechanisms
Chairperson: D.C. Baulcombe, The Sainsbury Laboratory, Norwich, United Kingdom

B. Bartel, Rice University, Houston, Texas: MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs.

D. Weigel, Max-Planck Institute for Developmental Biology, Tübingen, Germany: Specificity of plant microRNAs.

K. Barton, Carnegie Institution of Washington, Stanford, California: MicroRNA-mediated methylation of the class III HD-ZIP genes.

M. Timmermans, Cold Spring Harbor Laboratory: miRNA signals specify adaxial/abaxial leaf polarity.

J. Fletcher, USDA/University of California, Berkeley, Albany: MicroRNA regulation of *Arabidopsis* shoot apical meristem activity.

R. Scott Poethig, University of Pennsylvania, Philadelphia: Role for RNAi in the regulation of vegetative phase change in *Arabidopsis*.

SESSION 5: Defense, Counterdefense, and Posttranscriptional Silencing
Chairperson: M. Matzke, Austrian Academy of Sciences, Vienna

S. Huang, Monsanto Company, Mystic, Connecticut: Use of gene suppression to alter the amino acid composition of corn grain.

P. Green, University of Delaware, Newark: Novel approaches with potential for identification of small RNAs and target mRNAs.

T. Hall, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Size selective recognition of siRNA by an RNA silencing suppressor.

J. Burgyan, Agricultural Biotechnology Center, Godollo, Hungary: Molecular aspects of plant-virus-induced RNAi and suppression.

S.-W. Ding, Center for Plant Cell Biology, University of California, Riverside: miRNAs in viral pathogenesis.

V.B. Vance, University of South Carolina, Columbia: Dissecting the roles of HC-Pro and *Arabidopsis* dicer-like proteins in small RNA metabolism.

B. Ding, Ohio State University, Columbus: Viroid: A small noncoding RNA that traffics within a plant and alters developmental processes.

Summary: Moderator: J.C. Carrington, Oregon State University, Corvallis

J. Roberts, T. Adams, S. Huang

K. Barton

Origins and Evolution of the Nervous System

August 29–September 1

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **V. Hartenstein,** University of California, Los Angeles
I.A. Meinertzhagen, Dalhousie University, Halifax, Nova Scotia

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
V. Hartenstein, University of California, Los Angeles
I.A. Meinertzhagen, Dalhousie University, Halifax, Nova Scotia

SESSION 1: To Begin at the Beginning: CNS Component Molecules ("Nuts and Bolts")
Chairperson: P.A.V. Anderson, University of Florida, St. Augustine

H.C. Berg, Harvard University, Cambridge, Massachusetts: *E. coli* in motion: Physics of a single-celled nervous system.
R.W. Meech, University of Bristol, United Kingdom: Role of ion channels in sponge and jellyfish behavior: Minimalism and beyond.
B.M. Degnan, University of Queensland, Brisbane, Australia: Origins of the nervous systems: Insights from sponges and other basal metazoans.
P.A.V. Anderson, University of Florida, St. Augustine: Properties of the earliest nervous systems.

SESSION 2: Genes, Neural Patterning, and the Bilaterian Ancestor ("Stars and Stripes")
Chairperson: V. Hartenstein, University of California, Los Angeles

T. Gojobori, National Institute of Genetics, Shizuoka, Japan: Search for the evolutionary origin of the CNS: Comparative studies of gene expression in planarian and hydra neural cells.
V. Hartenstein, University of California, Los Angeles: *Drosophila* head structures compared with vertebrates: What does this reveal about the bilaterian ancestor?
H. Reichert, University of Basel, Switzerland: Origin of the tripartite bilaterian brain: Developmental genetic insights from *Drosophila*.
C.J. Lowe, University of California, Berkeley: The problems of reconstructing ancestral nervous systems from conserved patterning genes: Insights from hermichordates.
C. Nielsen, University of Copenhagen, Denmark: Apical organs and adult brains in protostomes and deuterostomes.
G. Scholtz, Humboldt University, Berlin, Germany: Levels of homology of nervous systems.

SESSION 3: Evolution of Cell Types: Genes for Neural Determination and Diversification ("Assembling the Team")
Chairperson: P. Lemaire, University of Marseille, France

L. Moroz, University of Florida, St. Augustine: Genomic approaches to the origin and evolution of the nervous system: Insights from molluscan neurogenomics.
P. Callaerts, University of Leuven, Belgium: Making neurons work: Insights from transcription factor–target gene studies.
D. Arendt, EMBL, Heidelberg, Germany: Tracing cell type diversification in nervous system evolution: A photoreceptor-centric view.
P. Lemaire, University of Marseille, France: Neural induction in chordates: An ancestral role for FGF as a neural inducer.
H. Wada, University of Tsukuba, Japan: Molecular evolutionary background for the innovation of the neural crest.

SESSION 4: The Chordate and Arthropod Progression ("Prometheus' Herald")
Chairperson: I.A. Meinertzhagen, Dalhousie University, Halifax, Nova Scotia

L. Holland, University of California, San Diego: Amphioxus and the evolutionary origins of the midbrain/hindbrain junction and neural crest.
S. Shimeld, University of Reading, United Kingdom: The evolutionary origin of vertebrate cranial sensory systems.
C. Ragsdale, University of Chicago, Illinois: Comparative molecular histology on the origins of the mammalian cerebral cortex.
N.J. Strausfeld, University of Arizona, Tucson: Using neural architectures to reconstruct evolutionary trajectories within the arthropods.
S. Harzsch, University of Ulm, Germany: Arthropod relationships: Linking brain architecture and phylogeny.
R.J. Greenspan, The Neuroscience Institute, San Diego, California: Arousal, attention, and the rudiments of consciousness in *Drosophila*.

SESSION 5: Evolution of Cells and Circuits "Nexus and Plexus"
Chairperson: M. Bate, University of Cambridge, United Kingdom

M. Bate, University of Cambridge, United Kingdom: Body plans and circuit diagrams for locomotor networks.
I.A. Meinertzhagen, Dalhousie University, Halifax, Nova Scotia: Cells, synapses, and circuits in the fly visual system: New circuits from old cells.
D.H. Paul, University of Victoria, Canada: How a real nervous system really evolved: Adding to, deleting from, and messing around with ancient neurobehavioral circuitry.
P.S. Katz, Georgia State University, Atlanta: Parallel, convergent, and divergent evolution of neural circuits in sea slugs.
D. Chklovskii, Cold Spring Harbor Laboratory: Why are neurons where they are? Why do neurons have the shape they do?

Overall General Discussion and Summary
Chairpersons: R.J. Greenspan, The Neuroscience Institute, San Diego, California; **H. Reichert,** University of Basel, Switzerland; **C. Nielsen,** University of Copenhagen, Denmark; **I.A. Meinertzhagen,** Dalhousie University, Halifax, Nova Scotia; **V. Hartenstein,** University of California, Los Angeles

H. Bourne, D. Paul

Integrating Disparate Data to Simulate Lymphocyte Function

September 19–22

FUNDED BY **Centers for Disease Control and Prevention**

ARRANGED BY **W.C. Reeves,** Centers for Disease Control & Prevention
S. Vernon, Centers for Disease Control & Prevention

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
S. Vernon, Centers for Disease Control & Prevention, Atlanta, Georgia

SESSION 1: Assessment of Normal Lymphocyte Function
Chairperson: U. Vollmer-Conna, University of New South Wales, Sydney, Australia

W.F. Hickey, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire: Leukocyte trafficking in the CNS: Communication between the brain and the body.

A. Shaw, Washington University, St. Louis, Missouri: Mediating lymphocyte function via the immunological synapse.

M. Dustin, New York University School of Medicine, New York: Integrins and lymphocyte function.

M. Gunzer, German Research Centre for Biotechnology, Braunschweig, Germany: Dynamic imaging of immune cell migration.

SESSION 2: Lymphocyte Function in Persistent Infection
Chairs: P.D. White, St. Bartholomew's Hospital, London, United Kingdom

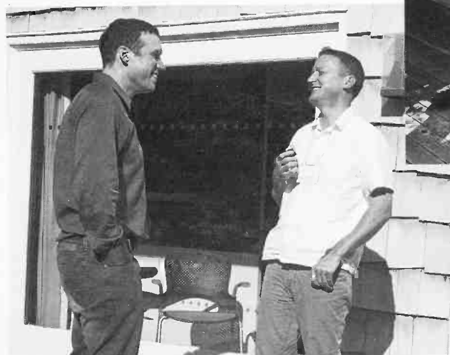
W.F. Hickey, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire: Effect of persistent infection on leukocyte trafficking and function in the CNS.

A. Shaw, Washington University, St. Louis, Missouri: The immunological synapse of persistently infected lymphocytes.

M. Dustin, New York University School of Medicine, New York: Effect of persistent infection on integrin-mediated T-cell function.

R. Taylor, University of Illinois, Chicago: Stress and lymphocyte function.

P.D. White, A. Lloyd



S. Efroni, E. Alakson

SESSION 3: Managing and Integrating Disparate Data
Chairperson: A. Lloyd, University of New South Wales, Sydney, Australia

A.K. Chakraborty, University of California, Berkeley: Integrating in silico and in vitro experiments to study how T lymphocytes communicate.

W. Tong, FDA's National Center for Toxicological Research, Jefferson, Arkansas: Integrating disparate data sources with data mining and visualization to facilitating toxicogenomics research

T. Wymore, Pittsburgh Supercomputing Center, Pennsylvania: Integrating structural bioinformatics and molecular simulation for the prediction of protein structure and function.

L. You, California Institute of Technology, Pasadena: Integrated understanding of biological networks by modeling.

S. Efroni, National Institutes of Health/NIAID, Bethesda, Maryland: Exploring emergent complexity: Reactive animation of thymocyte development.

SESSION 4: Computer Models of Living Cells
Chairpersons: E. Aslakson, Centers for Disease Control & Prevention, Atlanta, Georgia;
B. Gurbaxani, Center for Disease Control & Prevention, Atlanta, Georgia

B. Geortzel, Biomind LLC, Wheaton, Maryland: Application of probabilistic inference and machine learning to lymphocyte function.

A. Rundell, Purdue University, West Lafayette, Indiana: Mathematical modeling and analysis of T-cell signaling.

J. Fostel, National Center for Toxicogenomics, Research Triangle Park, North Carolina: Object models for system biology data.

S. Kumar, DARPA, Information Processing Technology Office, Arlington, Virginia: Bio-SPICE: An informatics and simulation tool for cell processes.

G. Broderick, University of Alberta, Canada: A parallel particle-based approach to whole-cell modeling.

SESSION 5: Brainstorming Session
Chairperson: S. Vernon, Centers for Disease Control & Prevention, Atlanta, Georgia

Discussion with facilitators

- Is simulation of a lymphocyte feasible?
- Should the focus of simulation be on a lymphocyte function?
- What are the pros and cons of simulated models?
- Does the current CFS immune data implicate a cell type or function that could be simulated?
- Is there a need for wet-lab work to augment our knowledge of lymphocyte function?
- If so, what is the most likely place to start?
- What are the (other) requirements?

Report by facilitators

Concluding Summary

Training and Education in Medical Genetics

October 20–22

FUNDED BY **Participant Funding**

ARRANGED BY **B.R. Korf,** University of Alabama, Birmingham

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

Welcome and Overview of Meeting:
G. Feldman, Wayne State University, Detroit, Michigan
B.R. Korf, University of Alabama, Birmingham
G. Wiesner, Case Western Reserve University, Cleveland, Ohio

Introductions
Brief Presentations

SESSION 1

C. Epstein, University of California, San Francisco: History and rationale for current approach to medical genetics training.

G. Wiesner, Case Western Reserve University, Cleveland, Ohio: Number of accredited MD Clinical Geneticists over history of ABMG.

G. Feldman, Wayne State University, Detroit, Michigan: Number of current accredited medical genetics residencies.

M. Blitzer, University of Maryland, Baltimore: Workforce study.

B.R. Korf, University of Alabama, Birmingham: Goals of physician training in medical genetics and roles of various types of professionals.

C. Epstein, V. Proud

SESSION 2

G. Wiesner, Case Western Reserve University, Cleveland, Ohio: Obstacles and challenges being faced in attracting trainees, supporting their training, and positioning them in the field.

G. Feldman, Wayne State University, Detroit, Michigan: Propose approaches to overcome the obstacles and challenges noted above.

Discussion

SESSION 3:
Action items and planned follow-up

Pandemic Disease Threat: Can We Develop a Global Vaccine Policy?

October 24–26

FUNDED BY **Albert B. Sabin Vaccine Institute, with the support of the Bill and Melinda Gates Foundation**

ARRANGED BY **V. Korn,** Albert B. Sabin Vaccine Institute
D.D. Mason, Albert B. Sabin Vaccine Institute
L.A. Miller, Intermedica, Inc.

Tri-Chairs: **D. Heymann,** World Health Organization
A. Osterhaus, Erasmus University Medical Center
L.A. Miller, Intermedica, Inc.

Distinguished Visiting Scholar: J.M. Barry, The Center for Bioenvironmental Research at Tuland and Xavier Universities, Washington, D.C.: The great influenza: The epic story of the deadliest plague in history.

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Conference Co-Chairs: **D.L. Heymann,** World Health Organization, Geneva, Switzerland
L.A. Miller, Intermedica, Inc., Darien, Connecticut
A. Osterhaus, Erasmus University Medical Center, Rotterdam, The Netherlands: Charge to the Conference.

Keynote Speaker: B. Schwartz, National Vaccine Program Office, Atlanta, Georgia: Global pandemic disease threats.

SESSION 1: Source Recognition and Surveillance
Chairperson: A. Osterhaus, Erasmus University Medical Center, Rotterdam, The Netherlands

Panel
A. Osterhaus, Erasmus University Medical Center, Rotterdam, The Netherlands
M. Miller, Fogarty International Center, NIH, Bethesda, Maryland
K. Stohr, World Health Organization, Geneva, Switzerland
M.T. Osterholm, University of Minnesota Academic Health Center, Minneapolis

M. Miller, Fogarty International Center, NIH, Bethesda, Maryland, and K. Stohr, World Health Organization, Geneva, Switzerland: What are we looking for?

A. Osterhaus, Erasmus University Medical Center, Rotterdam, The Netherlands, and M. Osterholm, University of Minnesota Academic Health Center, Minneapolis: What constitutes adequate surveillance?

SESSION 2: Planning and Response to Pandemics: Coordination and Feasibility
Chairperson: K. Stohr, World Health Organization, Geneva, Switzerland

Panel
J.T. Matthews, Aventis Pasteur, Swiftwater, Pennsylvania
D. Salisbury, Skipton House, London, United Kingdom
J. LeDuc, Centers for Disease Control and Prevention, Atlanta, Georgia,
T. Tam, Health Canada, Ottawa, Ontario
B. Schwartz, National Vaccine Program Office, Atlanta, Georgia
J. LeDuc, Centers for Disease Control and Prevention, Atlanta, Georgia
D. Salisbury, Skipton House, London, United Kingdom: Is a functional plan for a vaccine response strategy reasonable and how quickly could such a plan be developed?
K. Stohr, World Health Organization, Geneva, Switzerland
B. Schwartz, National Vaccine Program Office, Atlanta, Georgia: Who takes the lead: How and why?
J.T. Matthews, Aventis Pasteur, Swiftwater, Pennsylvania, and T. Tam, Health Canada, Ottawa, Ontario: How do we involve industry in pandemic preparedness?

Open Discussion and Consensus

Recommendations

SESSION 3: Achieving Capacity and Forecasting Demand
Chairperson: D.L. Heymann, World Health Organization, Geneva, Switzerland

Panel
S. Jadhav, Serum Institute of India, Ltd., Pune, India
I. Raw, Instituto Butantan, San Paulo, Brazil
L.K. Gordon, VaxGen, Inc., Brisbane, California: Large-scale vaccine production.
S. Jadhav, Serum Institute of India, Ltd., Pune, India
L.K. Gordon, VaxGen, Inc., Brisbane, California: Large-scale vaccine production.
I. Raw, Instituto Butantan, San Paulo, Brazil: How can the developing countries manage if there is a pandemic?

Addressing Regulatory and Liability Concerns

Open Discussion and Consensus

Recommendations

SESSION 4: Political and Economic Considerations on a Global Scale
Chairperson: J. LeDuc, Centers for Disease Control and Prevention, Atlanta, Georgia

Panel
C. Kang, NIH Korea, NIC Korea, Seoul, Korea
M.A. Chafee, The Harbour Group, Washington, D.C.
O. de Oliva, Pan American Health Organization, Washington, D.C.
S. Hall, UNICEF Plads, Copenhagen, Denmark
O. de Oliva, Pan American Health Organization, Washington, D.C.
C. Kang, NIH Korea, NIC Korea, Seoul, Korea: Building a plan inclusive of developing countries.
S. Hall, UNICEF Plads, Copenhagen, Denmark
M.A. Chafee, Harbour Group, Washington, D.C.: Establishing political commitment (disclosure, aid, etc.)

Open Discussion and Consensus

Recommendations

Conference Wrap Up/Next Steps from Here
L.A. Miller, Intermedica, Inc., Darien Connecticut

L.A. Miller, D. Heymann, J. Barry, D. Mason

The Biology of Neuroendocrine Tumors

October 26–29

FUNDED BY **The Verto Institute**

ARRANGED BY **A.J. Levine,** Institute for Advanced Study
E. Vosburgh, Verto Institute

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
E. Vosburgh, Verto Institute, Stamford, Connecticut: Verto activities to date.

SESSION 1: Genetics and Epigenetics in Neuroendocrine Tumors
Chairperson: A.J. Levine, Institute for Advanced Study, Princeton, New Jersey

C. Harris, Verto Institute, Princeton, New Jersey: The potential role of LINE1 retrotransposons in genomic instability of human tumors.

A. Levine, Institute for Advanced Study, Princeton, New Jersey: SNPS in the *p53* pathway.

A. Rashid, MD Anderson Cancer Center, Houston, Texas: Genetic and epigenetic alterations in neuroendocrine tumors.

R.V. Lloyd, Mayo Clinic, Rochester, Minnesota: Epigenetic regulation of protein expression in carcinoid tumors.

SESSION 2: Whole-genome Studies of Neuroendocrine Tumors
Chairperson: M.L. Meyerson, Dana Farber Cancer Institute, Boston, Massachusetts

B.T. Teh, Van Andel Research Institute, Grand Rapids, Michigan: Genetic studies of HPRT2 and its clinical implications.

D.C. Chung, Massachusetts General Hospital, Boston: Gene expression arrays in pancreatic neuroendocrine and GI carcinoid tumors.

P. Dahia, Dana Farber Cancer Institute, Boston, Massachusetts: Genome-wide approaches to a hereditary tumor model: potential for gene discovery and identification of novel signaling interactions in pheochromocytomas.

M.L. Meyerson, Dana Farber Cancer Institute, Boston, Massachusetts: Systemic genome analysis of cancer by SNP arrays and exon sequencing.

SESSION 3: Animal Models of Neuroendocrine Tumors
Chairperson: J.A. Epstein, University of Pennsylvania, Philadelphia

S.K. Kim and S. Karnik, Stanford University School of Medicine, California: Disrupted islet cell growth and differentiation in mouse genetic models.

K. Pietras, University of California, San Francisco: A multitargeted, metronomic, and MTD "chemo-switch" regimen is anti-angiogenic, producing objective responses

and survival benefit in a mouse model of neuroendocrine cancer.

Y. Xiong, University of North Carolina, Chapel Hill: *p18* double mutants.

T. Look and J. Kanki, Dana Farber Cancer Institute, Boston, Massachusetts: Targeted expression of MYCN selectively causes pancreatic neuroendocrine tumors in transgenic zebrafish.

Discussion: Key Points from the Day

A.J. Levine, Institute for Advanced Study, Princeton, New Jersey, and **Evan Vosburgh,** Verto Institute, Stamford, Connecticut

SESSION 4: Clinical Updates
Chairperson: J. Yao, Gastrointestinal Medical Oncology, Houston, Texas

M.H. Kulke, Dana Farber Cancer Institute, Boston, Massachusetts: Targeted therapies in the treatment of neuroendocrine tumors.

J. Yao, Gastrointestinal Medical Oncology, Houston, Texas: Developing targeted strategies for neuroendocrine carcinoma.

L. Kvols, University of South Florida, Tampa: An update on radiolabeled peptides as therapy for carcinoid and islet cell carcinomas.

Group discussion: Is there a need for a U.S.-based cooperative group for carcinoid and NET?

SESSION 5: Neuroendocrine Cell Biology
Chairperson: S.K. Kim, Stanford University School of Medicine, California

M.L. Meyerson, Dana Farber Cancer Institute, Boston, Massachusetts: Menin is associated with a histone methyltransferase complex.

X. Hua, University of Pennsylvania, Philadelphia: Regulation of apoptosis by menin.

S.K. Kim, Stanford University School of Medicine, California: Regulators of islet cell growth and differentiation.

SESSION 6: Carcinoid Tumor Biology
Chairperson: R.V. Lloyd, Mayo Clinic, Rochester, Minnesota

R.V. Lloyd, Mayo Clinic, Rochester, Minnesota: EGFR studies in carcinoid tumors.

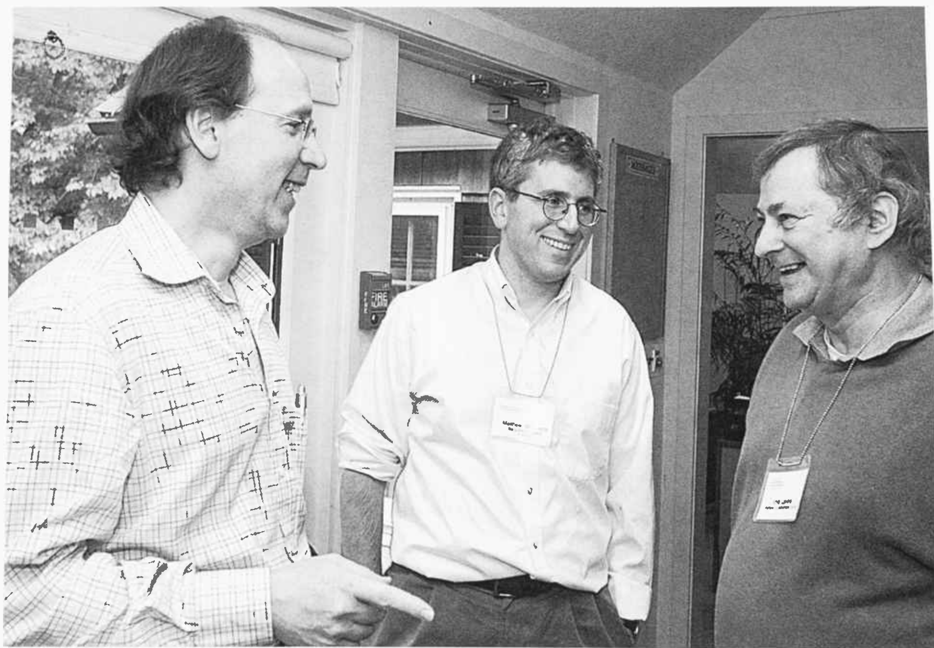
G. Friberg, University of Chicago, Illinois: c-*met*, p-*met* status of carcinoid tumors.

M.H. Kulke, Dana Farber Cancer Institute, Boston, Massachusetts: VEGF, VEGFR, EGFR, c-Kit, P-Kit, and CD31 studies of carcinoid tissue microarrays.

M. Essand, Uppsala University, Sweden: Gene expression in midgut carcinoid tumors: Potential targets for immunotherapy.

F. Leu, Verto Insititute, Princeton, New Jersey: Antibodies to extracellular region of hSSTR antibodies1-5.

SESSION 7: Review and Group Discussion led by Session Chairs



J. Sackler, M. Meyerson, A. Levine

Chromatin Remodeling and Gene Expression in Male Germ Cells

November 14–17

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **P. Sassone-Corsi,** Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
P. Sassone-Corsi, Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France

SESSION 1: Nuclear Organization

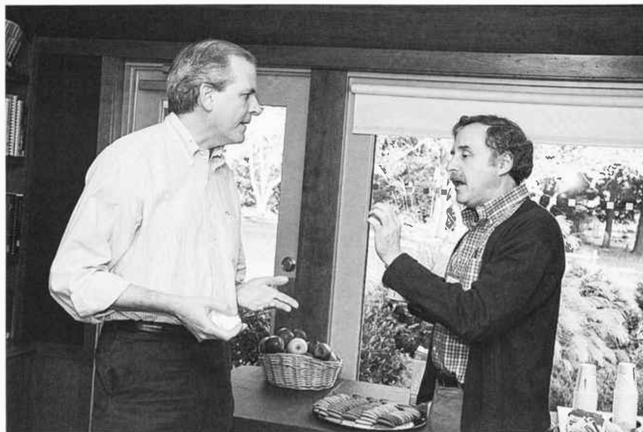
D. Spector, Cold Spring Harbor Laboratory: An overview of nuclear organization.
R. Camerini-Otero, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland: The mammalian sex body: What is required to form it and what is its role on the evolution of the X chromosome?
P. Moens, York University, Toronto, Canada: Immunocytological and FISH analysis of meiotic chromosome core, their associated proteins, and chromatin loop organization.

SESSION 2: Signaling

M. Matzuk, Baylor College of Medicine, Houston, Texas: Chromatin and germ-cell biology.
R.E. Braum, University of Washington, Seattle: Androgen regulation of mammalian spermatogenesis.

SESSION 3: Chromatin and Epigenetics

S. Henikoff, Fred Hutchinson Cancer Research Center, Seattle, Washington: Histone variants and nucleosome assembly pathways.
A. Nussenzweig, National Cancer Institute, NIH, Bethesda, Maryland: Chromatin remodeling mediated by histone H2AX
I. Davidson, Institut de Genetique et de Biologic Moleculaire et Cellulaire, Illkirch, France: Specialization of the general transcription apparatus and chromatin components in male germ cells.
M.L. Meistrich, University of Texas/M.D. Anderson Cancer Center, Houston: Abnormal chromatin remodeling in transition protein knockout mice.
P.S. Burgoyne, MRC National Institute for Medical Research, London, United Kingdom: The modulation of *X* and *Y* gene in meiosis and spermiogenesis in the mouse.



T. Bestor, S. Henikoff

SESSION 4: Epigenetics and Meiosis

P. Sassone-Corsi, Institut de Genetique et de Biologic Moleculaire et Cellulaire, Illkirch, France: Signaling through aurora kinases in germ cells.

C. Hoog, Karolinska Institutet, Stockholm, Sweden: Organization of the mammalian meiotic chromosome axes.

J. Schimenti, Cornell University, Ithaca, New York: Novel meiotic mutants in mice recovered by random mutagenesis.

T.H. Bestor, Columbia University: Irreversible gene silencing in the male germ line.

J. Trasler, Montreal Children's Hospital Research Institute, Montreal, Canada: Establishing, maintaining, and perturbing DNA methylation patterns in the male germ line.

SESSION 5: Mechanisms of Gene Expression

M.A. Handel, The Jackson Laboratory, Bar Harbor, Massachusetts: Chromatin correlates of meiotic transcriptional activity.

M.T. Fuller, Stanford University School of Medicine, California: Regulation of the primary spermatocyte transcription program by tissue-specific TAFs.

M. Wilkinson, University of Texas/M.D. Anderson Cancer Center, Houston: Alternative promoters insulated by tissue-specific DNA methlation boundaries.

D.J. Wolgemuth, Columbia University Medical Center: Mutation of the testis-specific bromodomain-containing gene *Brdt* results in defects in the differentiation of spermatid nuclei and male sterility.

H. Lin, Duke University Medical Center, Durham, North Carolina: Role of Piwi/Argonaute family proteins in gametogenesis.

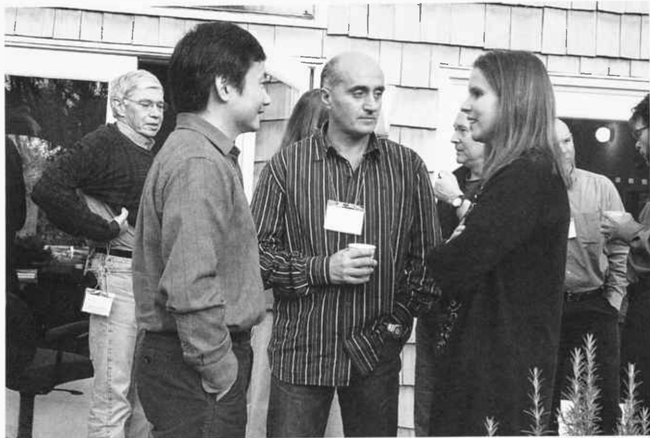
SESSION 6: Repair Systems

A. Grootegoed, Erasmus University Rotterdam, The Netherlands: Ubiquitin ligase Rad18Sc to the XY body and to other chromosomal regions that are unpaired and transcriptionally silenced in male meiotic prophase.

A. Shinohara, Osaka University, Japan: Role of Rad6-Bre1-mediated histone H2B ubiquitylation in the formation of double-strand breaks during meiotic recombination.

D. Georgia De Rooij, Utrecht University, The Netherlands: Repair gene expression during meiosis and spermatogenic arrests in deficient animals.

Review and Group Discussion



M. Meistrich, H. Lin, P. Sassone-Corsi, N. Kortaja

New Insights into Viral Disease from Mathematical Modeling of Biological Systems

December 5–8

FUNDED BY **Institute for Comparative Genomics**

ARRANGED BY
R. Breeze, Institute for Comparative Genomics
L. Horn, Institute for Comparative Genomics
W. Laegreid, USDA Agricultural Research Service
D. Rock, USDA Agricultural Research Service

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
R. Breeze, Institute for Comparative Genomics, Washington, D.C.: Why this meeting?

SESSION 1: Problems in Viral Pathogenesis
Chairperson: D.L. Rock, University of Connecticut, Storrs

A. Alcami, Centro Nacional de Biotecnologia, Madrid, Spain
B.L. Jacobs, Arizona State University, Tempe
P.B. Jahrling, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland
G. Letchworth, USDA, Agricultural Research Service, ABADRL, Laramie, Wyoming
E.S. Mocarski, Stanford University School of Medicine, California

Group Discussion

SESSION 2: Modeling Biological Systems
Chairperson: W.D. Wilson, Lawrence Livermore National Laboratory, California

H. Bolouri, Institute for Systems Biology, Seattle, Washington
P. Ghazal, University of Edinburgh Medical School, United Kingdom
P.C. Johnson, Icoria, Inc., Research Triangle Park, North Carolina
J. Rose, University of South Carolina, Columbia
C.M. Schaldach, Lawrence Livermore National Laboratory, California
W.D. Wilson, Lawrence Livermore National Laboratory, California

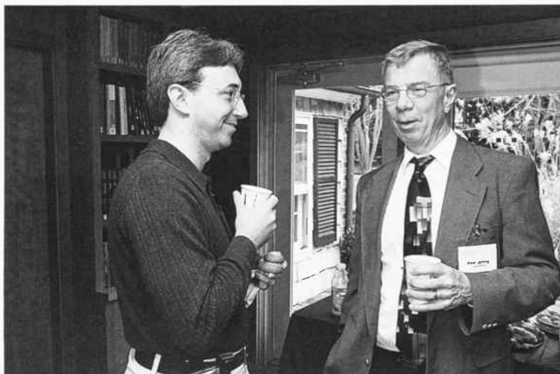
Group Discussion

SESSION 3: Modeling Virus Systems
Chairperson: W. Laegreid, Agricultural Research Service, Clay Center, Nebraska

R.A. Arnaout, Brigham & Women's Hospital, Harvard Medical School, Chestnut Hill, Massachusetts
R. Asquith, Imperial College, London, United Kingdom
N.M. Ferguson, Imperial College London, United Kingdom
A. Perelson, Los Alamos National Laboratory, New Mexico
R.J. Srivastava, University of Connecticut, Storrs
L. Weinberger, Berkeley, California

SESSION 4: Discussion of Research Needs/Opportunities/Challenges

SESSION 5: The Way Forward
Chairperson: R. Breeze, Institute for Comparative Genomics, Washington, D.C.



C. Cooke, P. Jahrling

Bioinformatic Strategies for the Epigenome

December 12–15

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **D.P. Barlow,** Center of Molecular Medicine GmbH of the Osterreischische Akademie der Wissenschaften
R. Martienssen, Cold Spring Harbor Laboratory

INTRODUCTION: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
D.P. Barlow, Center of Molecular Medicine GmbH of the Osterreischische Akademie der Wissenschaften and
R. Martienssen, Cold Spring Harbor Laboratory: Goals of Meeting: What are the key issues for integrating epigenetic data and bioinformatics?

SESSION 1
Chairperson: D.P. Barlow, Center of Molecular Medicine GmbH of the Osterreischische Akademie der Wissenschaften

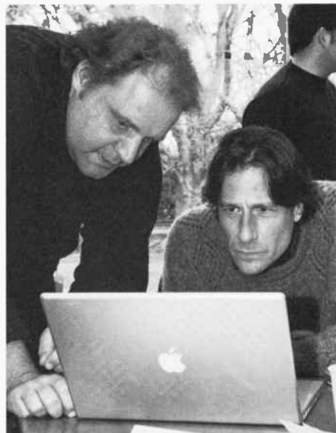
R. Martienssen, Cold Spring Harbor Laboratory: Transposons, tandem repeats, and small interfering RNA in *Arabidopsis* heterochromatin.
S. Beck, The Sanger Institute, Cambridge, United Kingdom: The human epigenome project: The pilot.
S. Eddy, HHMI/Washington University School of Medicine, St. Louis, Missouri: Computational methods for noncoding RNA structure and sequence analysis.
J. Walter, Saarland University, Saarbrucken, Germany: Comparative genomics and epigenomics.
X. Cheng, Emory University School of Medicine, Atlanta, Georgia: The chemistry of methylation: How many methyl groups are needed?
L. Joshua-Tor, Cold Spring Harbor Laboratory: Argonaute: The secret life of Slicer.
A. Neuwald, Cold Spring Harbor Laboratory: Using statistically inferred evolutionary constraints on protein sequences to predict epigenetic mechanisms.

SESSION 2
Chairperson: T.R. Gingeras, Affymetrix, Santa Clara, California

L. Ringrose, University of Heidelberg, Germany: Bioinformatic prediction of polycomb response elements in flies and vertebrates.
H. Stunnenberg, University of Nijmegen, The Netherlands: Functional genomics: Deciphering gene regulatory networks by Mass Spec and ChIP-chip.
J. Lieb, University of North Carolina, Chapel Hill: Nucleosome dynamics and transcription factor target selection in yeast.
B.D. Dynlacht, New York University School of Medicine, New York: Understanding mammalian cell cycle control and differentiation on a genome-wide scale.
G. Karpen, University of California, Berkeley: Epigenetics and chromosome functions.
W.G. Kelly, Emory University, Atlanta, Georgia: Homology pairing effects, genome defense, and sex chromosome evolution in *C. elegans*.

SESSION 3
Chairperson: S. Eddy, HHMI/Washington University School of Medicine, St. Louis, Missouri

T.H. Bestor, Columbia University, New York: Global structure of genomic methylation patterns.
R. Lucito, Cold Spring Harbor Laboratory: Microarray-based approaches to global methylation detection.



J. Walter, G. Karpen

J. Jurka, Genetic Information Research Institute, Mountain View California: CpG decay in *Alu* repeats: Germ-line-specific differences.

H.H. Kazazian, University of Pennsylvania School of Medicine, Philadelphia: Biology of mammalian retrotransposons.

S.R. Wessler, University of Georgia, Athens: Genome-wide analysis of transposable element-mediated alterations in rice gene expression.

R.W. Doerge, Purdue University, West Lafayette, Indiana: Mapping gene regulation: QTLs and microarrays.

T.R. Gingeras, Affymetrix, Santa Clara, California: Evidence of hidden transcriptome: Architecture and possible regulatory region.

SESSION 4

Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

V. Colot, Unite de Recherche en Genomique Vegetale, Cremieux, France: Building *Arabidopsis* epigenomic maps.

A. Ferguson-Smith, University of Cambridge, United Kingdom: Epigenetic features of a 2-Mb imprinted domain in mouse: A small piece of a bigger picture.

B. van Steensel, Netherlands Cancer Institute, The Netherlands: Chromatin mapping by DamID in flies and humans.

S. Kurdistani, University of California School of Medicine, Los Angeles: Mapping global patterns of histone acetylation to gene expression.

J. Martens, Research Institute of Molecular Pathology, Vienna, Austria: An epigenetic map of the mouse genome.

D.P. Barlow, Center of Molecular Medicine GmbH of the Osterreischische Akademie der Wissenschaften: ChIP on Chip histone profiling using mouse genome path arrays.

Discussion: Global epimapping strategies technical applications.

SESSION 5

Chairperson: H.H. Kazazian, University of Pennsylvania School of Medicine, Philadelphia

L.D. Stein, Cold Spring Harbor Laboratory: Database strategies for interconnecting biological pathways with the genome.

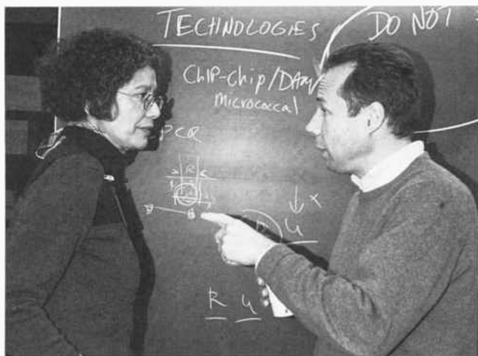
N. Brockdorff, Hammersmith Hospital, London, United Kingdom: Development of a genome environment browser for linking epigenetics and genomics.

R. Jorgensen, University of Arizona, Tucson: The Plant Chromatin Database: Integrating information on plant chromatin components and complexes.

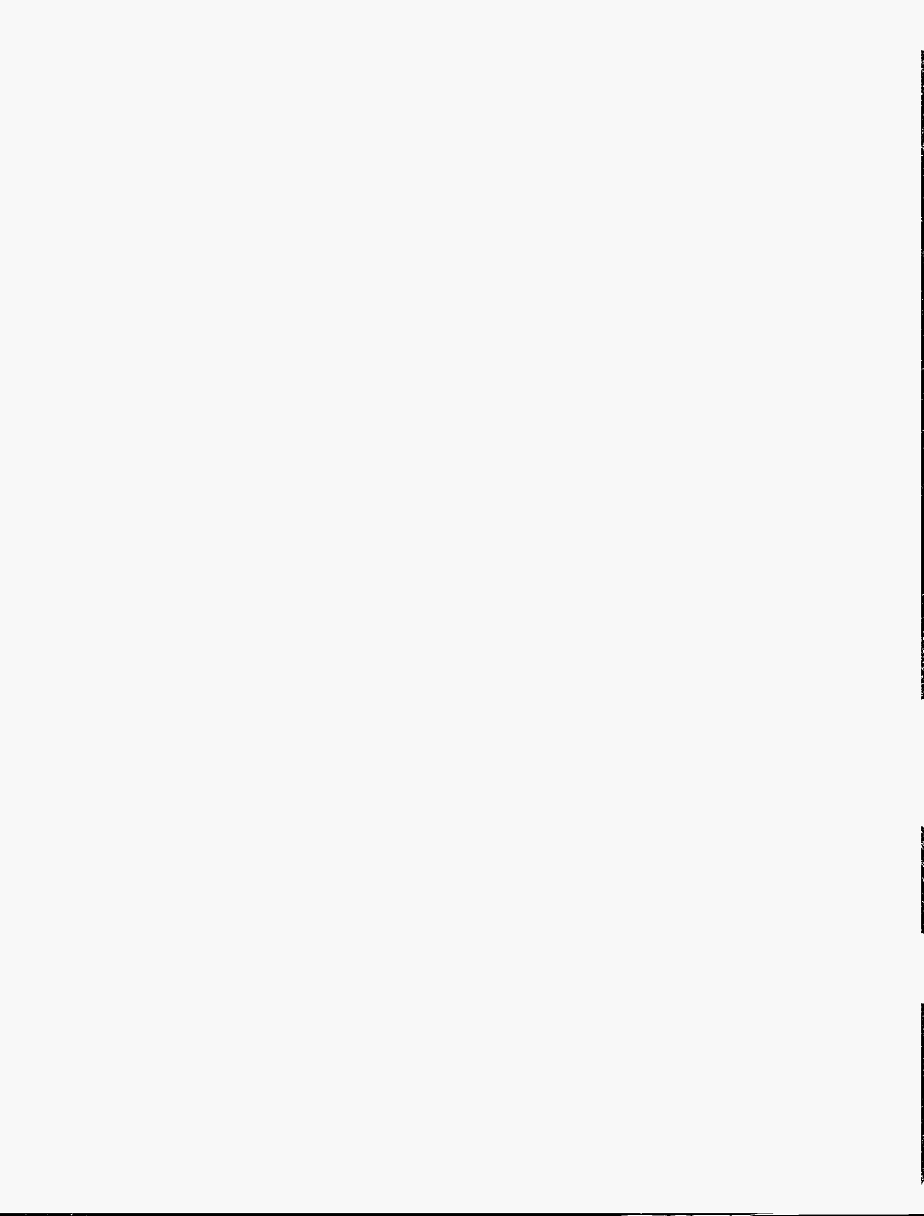
M. Vaughn, Cold Spring Harbor Laboratory: Mapping the epigenotic potential of *Arabidopsis*.

Summary/Further Action

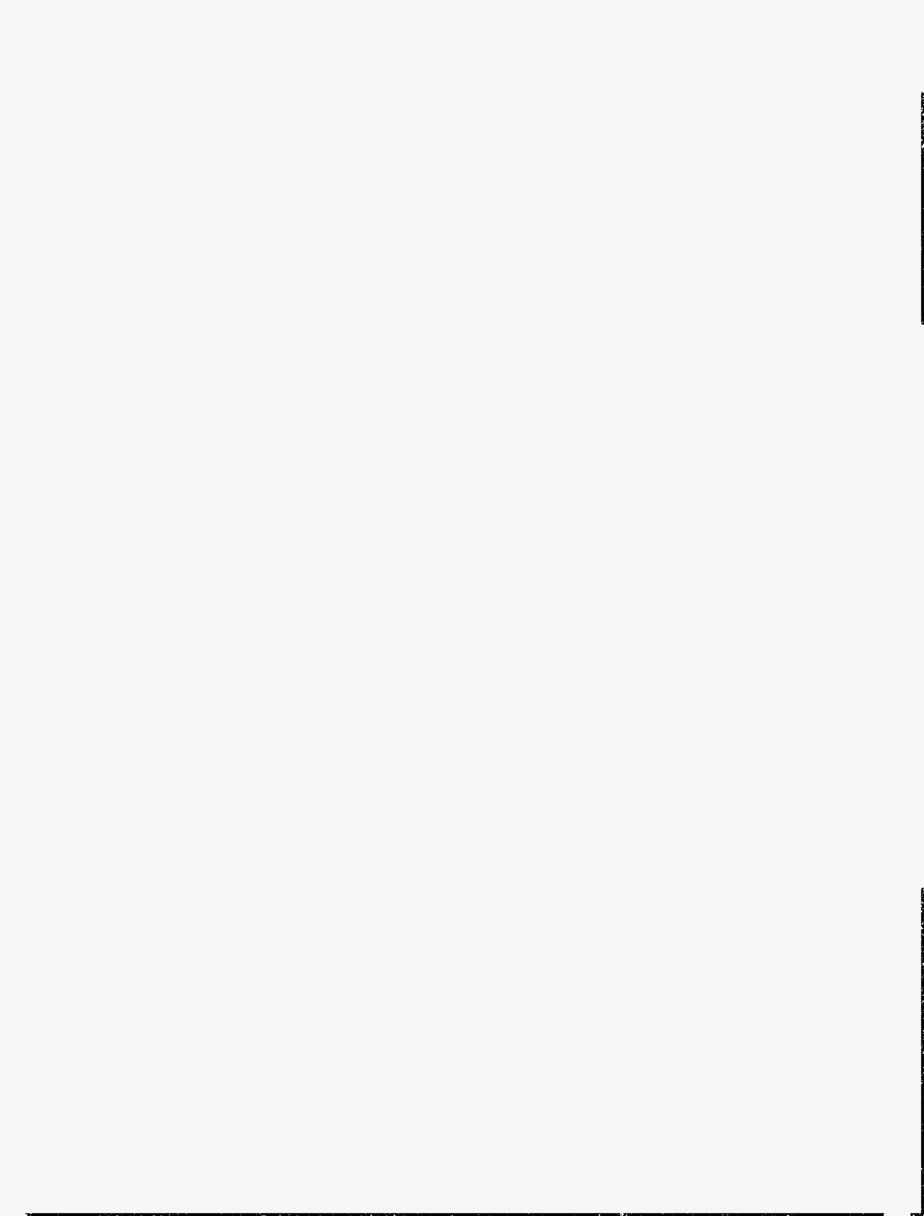
Moderators: D.P. Barlow, Center of Molecular Medicine GmbH of the Osterreischische Akademie der Wissenschaften; **R. Martienssen,** Cold Spring Harbor Laboratory



D. Barlow, V. Colot



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
Judy Cumella-Korabik	Tracy Behar	Shirley Chan	Adrian Arva
Nancy Daidola	Elna Carrasco	Eun-Sook Jeong	Tom Bubulya
Mary Lamont	Jeanette Collette	Susan Lauter	Uwe Hilgert
David Micklos	Erin Maroney	Chun-hua Yang	Craig Hinkley
Karen Orzel	Amanda McBrien		
Carolyn Reid	Lauren Weidler		

If you needed expert advice on a rare tumor, you would contact the most prominent physician you know. This physician would put you in contact with the most prominent oncologist he/she knows, who, in turn, would put you in contact with the expert you seek. Each of these well-connected individuals is a node in a social network that positions you just four steps from the critical information you need.

This is the premise of the play *Six Degrees of Separation:* We live in a "small world" in which only several social connections separate any two people. Social science research has, indeed, upheld the notion that it takes only a handful of acquaintances to relay a letter to anyone in the United States or an e-mail to anyone in the world! The small-world principle organizes activities on many levels of complex systems. Cells, the basic units of biological function, are networks of communicating molecules. For example, each of the several hundred molecules involved in processing a nervous signal is, on average, separated by less than four connections from any other molecule.

By the same token, people harness the small-world aspect of the Internet—hopping between prominent nodes to quickly locate information about even the most arcane topic. During the past 18 months, we developed the concept for a novel Internet site on modern neuroscience research that is built on this small-world principle. We envisioned a nonlinear "knowledge network" where one can easily move from one piece of information to another through a minimum number of connections.

Public Education in a Revolutionary Science

So we were thrilled when the Dana Foundation announced in October that it would provide $1 million to support the project over 4 years. In a sense, the grant follows up on my 1990 Dana Award for Pioneering Achievement in Education.

The site will be developed in parallel to research emanating from the *Genes to Cognition* (*G2C*) Program at the Wellcome Trust Sanger Institute in Cambridge, England. *G2C* program director Seth Grant, who did postdoctoral work at CSHL, is using network theory to study the gene interactions that result in human thinking and disorders of thinking. This research base will be expanded with insights drawn from CSHL scientists, the neuroscience start-up company Helicon Therapeutics, and neuroscience initiatives supported by the Dana Foundation.

Science education and public outreach typically begin well after a "scientific revolution" has settled down into what Thomas Kuhn called "normal science"—resulting in a set of facts that can be conveniently categorized and presented as unchallenged dogma. Rather than presenting science as a completed endeavor, with nothing important left to discover, we want to involve *G2C Online* users in this revolutionary period of neuroscience research. We want them to be online when new insights into human memory and new treatments for cognitive disorders appear on the horizon.

Thinking about the Biology of Thought

G2C Online essentially will be a three-dimensional, multimedia concept map of the science of the human brain, allowing site users to explore the relationships among concepts and to assess their own learning. In mimicking both the molecules of thought (and resulting human social behaviors), the site will provide users with a real life exercise in "metacognition"—thinking about the biology of thought.

The goal of *G2C Online* is to help people explore the relationship among genes, thinking human behavior, and cognitive and behavioral disorders. Content at the site will embody several key concepts of modern neuroscience:

- Cognition arises from a "thinking machine" whose parts can be understood at different levels of complexity—from anatomical structures of the brain, to neural circuits, to molecular signals.
- Neurons and molecules work together in redundant networks that allow rapid communication through key nodes and can compensate for loss of individual components.
- Cognitive information is encoded in patterns of nervous activity and decoded by molecular listening devices at the junction between nerve cells (the synapse).
- The molecules of learning and memory have been conserved by evolution, allowing scientists to model cognitive processes in simple organisms.
- Although humans inherit genes that set the parameters of learning and memory, the cognitive machinery is continuously altered by experience.
- Cognitive disorders are caused by discrete changes in genes and proteins that can potentially be targeted by therapeutic molecules.
- Understanding human cognition has ethical and social consequences; the eugenics programs of the 20th century illustrate the tragic outcome of misguided efforts to limit the spread of "unfit" behaviors.

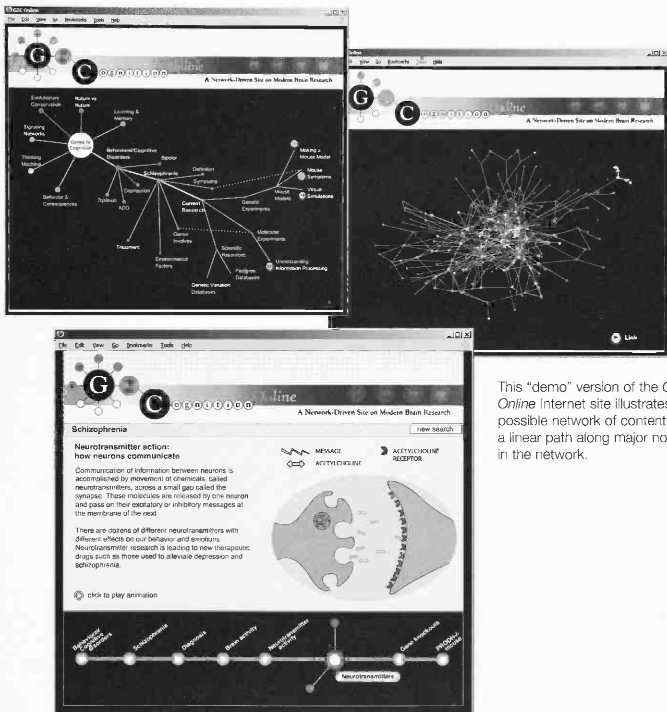
Building a "Knowledge Network"

The computer technology behind *G2C Online* will be every bit as revolutionary as the science it covers. Each content item—and its relationships to other items—will be stored in a database. A "network engine" will draw items from the database to construct a network customized according to the preferences and knowledge level of the user. Rich multimedia modules will form the major nodes of the network. Key among these materials will be narrated animations, which seamlessly integrate video narration with cell and molecular animations, experiment simulations, and bioinformatics tools.

The site may be used in a linear or nonlinear mode. In the linear mode, one will be able to follow a predetermined path prepared by the editorial team or a path submitted by another user. A person following a linear path will see their course ahead and behind, but may also deviate to explore adjacent nodes. In the nonlinear or exploratory mode, one will enter the network by selecting a node of interest and then follow connections out from that node. Alternatively, one can identify a node or set of nodes that matches terms entered in a search engine. The distribution of these nodes in the graphical view of the network can provide visual clues to locate other relevant content.

To simplify navigation, a selected path will be extracted from the network and displayed as a "subway" map showing only the "stations" (major nodes). Movement along the "subway line" will be tracked, and outward excursions (minor nodes) will be displayed upon arrival at each "station." A database engine will allow site users to generate a view of the network from any point, chart a course through the network, and store a course for analysis or modification.

We envision three target audiences for *G2C Online:* biology students, psychology students, and families who are facing mental health problems. Students and teachers are the DNALC's natural constituency; online surveys show that they make use of DNALC Internet sites for authoritative information



This "demo" version of the *G2C Online* Internet site illustrates a possible network of content and a linear path along major nodes in the network.

about a variety of class assignments and projects. By targeting the site to the "bright teenager," materials will be at a level of science comprehensible to a broad slice of the adult population. Therefore, the second major audience will be families who are facing mental health problems. Many families dealing for the first time with a behavioral or cognitive disorder do not know where to turn for authoritative answers to their questions. Others wish to follow up on cursory information presented by health care providers. This is the audience that we have been specifically building with *Your Genes, Your Health* (www.ygyh.org), our multimedia site on common genetic disorders.

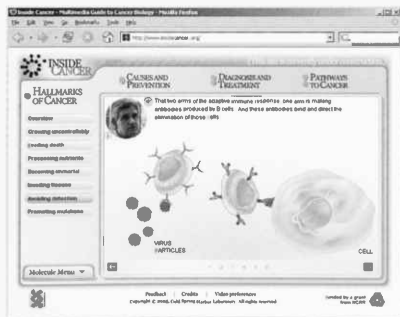
The ability to generate custom entry points and views will allow us to design a unique experience for each audience. By selecting from a list of questions or information preferences, a user may generate an optimized network and suggested path to follow. So, educational audiences might enter the site through major nodes that address key content standards and teaching syllabuses (for example, Advanced Placement biology or psychology), whereas health care audiences might enter the site by way of individual diseases or common questions about cognitive disorders. As a result, an Advanced Placement psychology student will be provided a substantially different network to browse than will a family member interested in autism. Each user will be provided logical "anchor" points from which to enter the knowledge network and from which to launch further explorations.

Inside Cancer Readied for Launch in 2005

When we initiated the *Inside Cancer* project under an NIH Science Education Partnership Award, in 2001, there was no effective way to integrate different media types—such as video, animation, and flat art—into a seamless Web page. The technology for achieving this level of media integration, *Flash MX* by Macromedia, first became available in the summer of 2002. During 2002–2003, the *Biomedia* Group tested the large-scale application of the new *Flash* technology during the construction of *DNA Interactive*. Equipped with this new expertise, in 2003, we resolved to redevelop *Inside Cancer* in *Flash MX*. This decision to substantially upgrade the *Inside Cancer* site required scrapping an earlier HTML design and retooling content—with the result of throwing production behind schedule.

As 2004 drew to a close, the site was more than 90% complete. We think our audience will agree that it is one of the most advanced science content sites on the Internet and that we were justified in waiting for the right media integration technology. Using dynamic animations, we have integrated technology, design, and content to present information that cannot be found in any other educational medium (including medical textbooks). In this unique format, one of 28 cancer experts interviewed for the project narrates an animation of cellular and molecular events, with his or her video appearing in a "bubble" analogous to the narrative of a cartoon. When formally launched in 2005, the four major sections of *Inside Cancer* will provide authoritative and engaging information on how knowledge of the cancer cell is changing cancer diagnosis and treatment:

- *Hallmarks of Cancer* follows a landmark paper of the same title, published in *Cell* (2000), that summarized the major molecular and cellular aspects of cancer. To our knowledge, *Inside Cancer* offers the first popular treatment of these key tenets of cancer genetics and cell biology. The section features clips from interviews with Douglas Hanahan and Robert Weinberg, the authors of the *Cell* article. In "Growing Uncontrollably," one can see the first example of fully integrated video and animation.

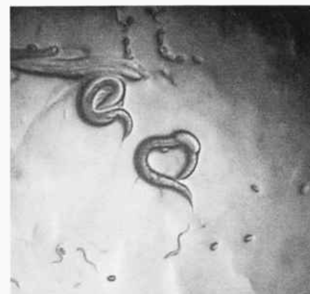


Inside Cancer features video narrations and animations in one screen. In the *Hallmarks of Cancer* section, Bruce Stillman, CSHL President, explains how cancer cells avoid detection by the body's normal immune response. In *Pathways to Cancer*, below, growth factor proteins are packaged into vesicles within a cell.

- *Causes and Prevention* uses epidemiological data to highlight behaviors and environmental factors that increase cancer risk. The overview first debunks the common misconception that inheritance and toxic chemicals are major causes of cancer. Then users are challenged to link potential causes of cancer to patterns of cancer incidence worldwide. Individual sections highlight how environment, behavior, and inheritance contribute to different major cancers—and how these insights relate to preventing disease. The influences of mold (afflatoxin), diet, viruses, and inheritance are explained in the context of the key cancers of the liver, prostate, cervix, and colon. Students also explore the events that spurred the rapid increase in cigarette smoking in the 20th century, which is almost wholly responsible for the continuing "epidemic" of lung cancer.
- *Diagnosis and Treatment* shows how cellular and molecular techniques are used to diagnose and tailor cancer treatment according to specific genetic changes in the patient's tumor. A section on pathology gives site users a chance to try their hand at distinguishing normal and tumor cells, as seen under the microscope, and to follow an endoscope through a patient's colon. In pharmacogenetics, narrated animations explain how cell receptors and gene profiling are used to tailor treatment for breast cancer patients. A section on targeted therapies explain how a new generation of drugs block specific molecules that conduct growth signals in the tumor cell—using the examples of Tamoxifen and Herceptin for breast cancer, and Gleevec for chronic myeloid leukemia.
- *Pathways to Cancer* is a high-quality, three-dimensional animation that follows a common signaling pathway through which growth commands are transmitted from cell surface to the nucleus. The tour is narrated by noted voice talent Doug Thomas. The section incorporates a "molecule menu" and freeze-frame rollovers that allow site users to learn more about the molecules that conduct a growth signal through the cell and how they are involved in oncogenesis.

NSF Grant to Develop New Laboratories on RNAi

Our effort to strengthen direct ties to CSHL research received additional affirmation in May when we received a $300,000 grant from the National Science Foundation (NSF) to develop new laboratories on RNA interference (RNAi) and functional genomics. This project follows the template of a project initiated last year to develop new experiments in plant genomics. Like the plant project, the RNAi project received unanimous "excellent" ratings from the five reviewers.



The *C. elegans* worm on the lower right illustrates the "blister" phenotype.

During the past 15 years, high school and college biology faculty have implemented key laboratories that illustrate basic concepts of microbial and molecular genetics. The completion of the Human Genome Project challenges these teachers to move laboratory instruction to a higher level of biological integration—the functional analysis of genes and proteins in eukaryotic organisms. Thus, we will develop a module of investigative laboratories and bioinformatics exercises that engage students in the new technologies of RNA-mediated genetic interference and computer-based genome analysis.

Until recently, targeted gene inactivation ("knock-out") or rescue ("knock-in") by homologous recombination required significant resources and was confined to research labs. RNAi offers a remarkably simple system in the roundworm *Caenorhabditis elegans*. Bacteria are transformed with a plasmid encoding "antisense" RNA to the worm gene of interest, and a lawn of the bacteria is grown on an agar plate. The RNAi response is triggered when worms are released on the agar plate and ingest the transformed bacteria expressing the antisense RNA. (Bacteria are a normal food source for *C. elegans*.) The effects of the loss of gene function are then observed in the offspring (either embryos or larvae) of hermaphrodite worms.

We believe that the RNAi/*C. elegans* experimental system is simple and robust enough to join bacterial transformation and gel electrophoresis as a mainstay of the high school and college teaching lab-

oratory. After learning basic RNAi techniques, students can make use of freely available resources to design their own experiments to explore the function of virtually any of the predicted 19,427 genes of *C. elegans*. The premise that RNAi labs may prove broadly useful in biology education led to unusual joint funding of the project by NSF's precollege and collegiate curriculum development programs.

The project is a collaboration among staff at the DNALC, faculty at 2- and 4-year colleges, and scientists in Greg Hannon's lab at CSHL, which pioneered RNAi technology. An Advisory Panel of college faculty from diverse geographic locations will be involved in all aspects of the project, including development and testing. Following a year of development, the experiments and computer exercises will be tested with 72 biology teachers at three sites around the United States. Instructional and bioinformatics resources developed in this project will be disseminated via the DNALC's Internet site, Cold Spring Harbor Laboratory Press, and Carolina Biological Supply Company.

National Science Foundation Fellowships

In summer 2004, two pairs of Faculty-Student Fellows spent 3 weeks at Cold Spring Harbor participating in the NSF-sponsored "Building Leadership to Expand Participation of Underrepresented Minorities in Plant Genetics and Genomics" program: Dr. Javier Gozalez-Ramos and Adriana Robbins of Texas A&M University, and Dr. Olga Kopp and Elise Unice of Utah Valley State College. The Fellows participated in a mix of activities at the Dolan DNALC and the Hazen Genome Sequencing Center. During the first week, Fellows conducted an integrated set of experiments from the NSF-sponsored *Greenomes* course. Designed for use in high school and college biology courses, these experiments use plant systems to illustrate major concepts of molecular and genomic biology, including the relationship between molecular genotype and phenotype, transposon mutagenesis, chromosome mapping, homeotic development, functional analysis, and transgene detection. These experiments have been specifically designed for ease of replication in high school and college teaching laboratories. In the fall, we began working with Dr. Ramos and Dr. Kopp to organize regional training workshops on *Plant Genetics and Genomics* to introduce the plant labs to high school and college faculty. The workshops—to be held in summer 2005—will be co-taught by the Fellows and DNALC staff.

NSF Fellows Bob Wheeler and Debra Burhans.

A second NSF-sponsored fellowship, "Building Leadership to Develop Educational Bioinformatics Tools in Plant Genomics," was a collaboration between the Dolan DNALC and Gramene, an Internet resource for comparative genome analysis of grasses. The main objective of this fellowship is to assist with the production of a *Student Genome Viewer* that will allow advanced high school and college classes to directly participate in the annotation of the rice genome. Two fellowship positions were awarded in summer 2004: Mr. Robert Wheeler from Pine Creek High School (Colorado Springs, Colorado) and Dr. Debra Burhans from Canisius College (Buffalo, New York).

The USDA also provided funds for fellowships at Cold Spring Harbor Laboratory for two underrepresented minorities in biological sciences. Cesar Gutierrez, a biology teacher at Jon H. Reagan High School in Austin, Texas and a lead teacher in the NSF-sponsored Bio-Link Center for Advanced Technological Education at Austin Community College, spent 2 weeks working with DNALC staff doing advanced development of the *Student Genome Viewer*. Paul De La Rosa, a biology student at the Univeristy of Texas, spent 1 week learning about DNA polymorphisms at the DNALC and 1 week learning large-scale sequencing techniques at the Hazen Genome Sequencing Center.

Pfizer Leadership Institute

In July, we completed the fourth *Leadership Institute* under support from the Pfizer Foundation. We had initiated this program in the early 1990s with support from the NSF, only to see it wither when the NSF shifted its focus to central school authorities. Pfizer support allowed us to reinstate what we believe is the most advanced activity to reward the nation's top biology teachers with a summer sojourn at Cold

Participants in the Pfizer *Leadership Institute* and the NSF Fellowship Program wrap up a 3-week stay in Cold Spring Harbor.

Spring Harbor. However, with the lapse of Pfizer funding in 2005, we will again need to search for support for this jewel in the DNALC's crown of training courses for biology faculty.

The 2004 Pfizer *Leadership Institute* participants represented a diverse range of backgrounds and experience. The Institute drew 18 faculty representing 12 different states. One third came from schools with high percentages of minority or disadvantaged students, and one third were from rural regions. The American faculty were joined by four teachers from Singapore, who visited as part of our ongoing collaboration with the Singapore Ministry of Education.

During their 3-week stay, the *Leadership* participants lived and breathed science—walking in the footsteps of Nobel Prize winners and taking meals with some of the brightest researchers in the world. The 2004 workshop focused on the study of human and plant genomes. All topics were addressed in a three-pronged approach of lectures/seminars, wet-labs, and computer work. Included in the lab work was the opportunity to test the DNALC's very latest lab protocols, in advance of their availability at other institutions or from commercial suppliers. The Institute curriculum also drew heavily on the Laboratory, with scientist seminars and visits to Uplands Farm agricultural field station and the Hazen Genome Sequencing Center. *Leadership* teachers were introduced to a number of computer-based utilities that collect, analyze, and display DNA data, including several developed at the DNALC. A tutorial held by the DNALC WWW designers introduced participants to technology that enabled them to create personal teaching environments.

Four days were dedicated to independent study or group projects to increase mastery and/or to adapt materials for classroom use. Projects included screening supermarket foods for genetic modification, sequencing dog mitochondrial DNA, mutating the "glow" gene for green fluorescent protein, and designing a polymerase chain reaction (PCR)-based method to examine polymorphisms in human olfactory receptor genes. Other teachers expanded computer-based studies on bioinformatics and Web page construction.

Genetic Origins

Genetic Origins is one of the most unique programs developed here at the DNALC. It consists of an integrated set of experiments, gratis DNA sequencing, and online tools that allow students to use their own DNA polymorphisms as the starting point for investigations on human relatedness, evolutionary history, and pharmacogenetics. *Genetic Origins* presents an accurate analog of human genome research, which incorporates the highest aspirations of "hands-on" learning. This program was initiated with federal grants, but has been maintained with DNALC cash flow during the last several years.

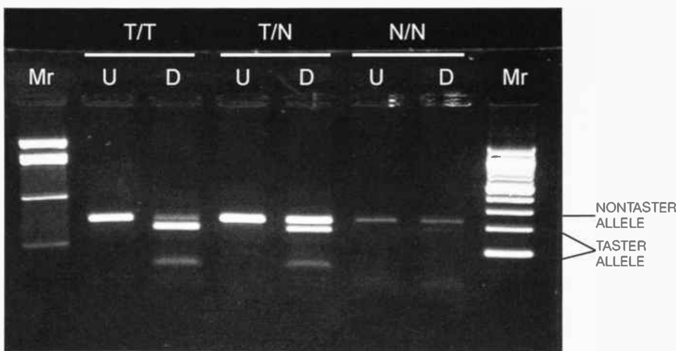
To maintain *Genetic Origins* as a free program to students around the United States and to make needed upgrades to the Internet components, we gratefully received a $50,000 grant from our friend and colleague Mike Gillman, Senior Vice President of Research at Biogen Idec, and a $10,000 grant from William A. Haseltine, President of the William A. Haseltine Foundation for Medical Science and the Arts.

The redeveloped sites will conform to simple rules for intuitive use: consistent and logical placement of tool bars, minimum use of submenus or "nested" screens, and minimal text. Redevelopment will make cost-effective use of templates developed for recent DNALC projects and will employ highly designed "skins" that give tools and media players the sense of being real onscreen objects. The *Genetics Origins* labs will be ported through a lab virtual lab notebook player similar to the *Greenomes* Internet site we are currently developing. The *Bioservers* workspaces and data functions will be repackaged in attractive skins in the manner of *Bioinformatics Calculator* and *Gene Boy*.

New Mammalian PCR Labs

Our repertoire of advanced PCR experiments on DNA polymorphisms was significantly strengthened in 2004. We added two new laboratories that demonstrate the use of single nucleotide polymorphisms (SNPs) in predicting phenotypes. Both experiments use a hybrid method termed amplified restriction-length polymorphism (ampliFLP), in which a PCR product is subsequently cut with a restriction enzyme. A restriction enzyme recognition sequence is incorporated into one PCR primer. Then, an SNP located at the restriction site is detected as a length polymorphism on an agarose gel.

SNPs and Human Taste examines an SNP in the bitter taste receptor (TAS2R) that correlates with the ability to taste phenylthiocarbamide (PTC). CSHL researcher Albert Blakeslee determined in 1932 that tasting the bitter PTC chemical is inherited in a dominant character. Since that time, it has been a classic demonstration of Mendelian inheritance in high school genetics classes. However, a new analysis, on which our experiment is based, shows that the inheritance of PTC tasting ability is not as simple as students have long been taught. Like many SNPs of medical importance, the SNP we test for combines with two other SNPs to produce complex combinations (haplotypes) that predict tasting ability—but not perfectly!



Human PTC polymorphism genotypes detected by restriction digest (D) of PCR products (U). Genotypes shown are taster/taster (T/T), taster/nontaster (T/N), and nontaster/nontaster (N/N).

Canine Ivermectin Sensititvity is real test of pharmacogenetic importance to dog owners who give their pets Ivermectin to control heartworm and other parasites. DNA is isolated from cheek swabs of pet dogs, amplified by PCR, and examined for an SNP in the *mdr1* (multidrug resistance) gene. Dogs that are homozygous for the *mdr1* mutation cannot metabolize Ivermectin and may have a toxic or fatal reaction. Collies, Shetland sheepdogs, Australian shepherds, and Old English sheepdogs are among the breeds most at risk for Ivermectin sensitivity.

New Visitor Highs in 2004

Visitation to the DNALC facility and to its family of Internet sites reached new highs in 2004. DNALC hosted 37,617 visitors, with cumulative visitation topping one-quarter million since our founding in 1988! Lab instruction increased 23%, to 27,142 students, with *DNALC West* allowing us to expand our reach in Nassau County and New York City. Of these students, 16,903 received instruction at the DNALC or *West* facilities, and 10,239 received instruction at their own schools. (It is worth noting that in-school instruction typically amounts to four class visits by a DNALC educator, so that the number of DNALC-taught labs is several times the number of students reported!)

These numbers keep us solidly in the lead of institutions that offer hands-on instruction in genetics and DNA for precollege students. Ironically, our next closest competitor is the Singapore Science Center, whose instructional program we helped develop under a partnership with the Singapore Ministry of Education. In 2004, its first full year of operation, the Science Center provided labs for 17,141 students, teachers, and members of the general public!

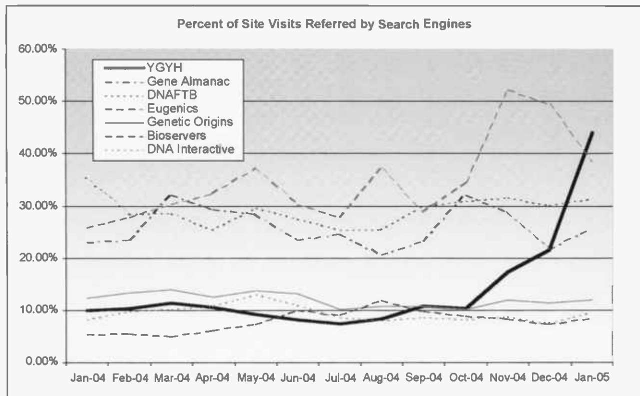
"Virtual" visitors to the family of Internet sites reached via the DNALC's portal, *Gene Almanac*, reached 5.44 million—an increase of 11% over 2003. Visitors spent an average of about eight minutes at the informational sites, *DNA Interactive* and *Your Genes, Your Health*, and about 10 minutes at our animated text, *DNA from the Beginning*. Visits averaged substantially longer at our historical resource, the *Image Archive on the American Eugenics Movement* (11.5 minutes) and *Bioservers* (16 minutes).

Internet Site	Average visit length (in minutes)	Visits in 2004	Increase from 2003 (%)
Gene Almanac	8:23	2,135,981	4.77
DNA from the Beginning	10:06	1,264,919	0.68
Your Genes, Your Health	8:48	860,583	15.46
DNA Interactive	8:04	650,096	72.23
Image Archive on the American Eugenics Movement	11:25	283,405	14.72
Bioservers	16:06	137,988	4.92
Genetic Origins	9:05	109,022	20.90
All Sites	**10:17**	**5,441,994**	**11.37**

A midyear analysis of our Internet traffic found that only about 10% of visits to *Your Genes, Your Health (YGYH)* resulted from referrals by Internet search engines. This was surprising to us, because this site contains information on individual genetic diseases that we knew was the subject of many searches by families and medical professionals. It then dawned on us that the multimedia content developed in Macromedia *Flash* was not "visible" to popular search engines, such as *Google*. "Spiders" and other indexers sent out by search engines could not find content contained in *Flash* multimedia files; thus, words in the text of animations and videos in several of most technologically advanced sites were not being found by relevant Internet searches!

In 2004, we found a solution to this problem. The text files for each animation and video were constructed into a skeleton of "meta" pages, linked to the homepage, which reflected each site's content

and architecture. As indexers revisited the sites, they detected the updated body of text and rebuilt comprehensive indexes that are now searchable. The results were dramatic. Within 2 months of implementing the meta-indexing scheme, searches rose sharply to account for more than 40% of visits to *YGYH*.



Learning the Effects of Our Summer DNA Camps

We began teaching intensive, week-long "camps" for students in the summer of 1985, when we offered *DNA Science* at the Wheatley School in Old Westbury. Since that time, more than 6700 middle and high school students have spent one week at one of seven camps offered each summer. Beginning with *Fun With DNA* in the fifth grade, a student can take a different workshop each summer—culminating with *DNA Science* and *Vertebrate Genomic Biology* in high school. Although we know that a number of graduates of the summer camp program become interns at the DNALC, we have long wondered about what the others do after taking one or more of our summer courses. So in 2004, we completed a major survey of students who took summer camps from 1990 to 2001.

We received completed surveys from 789 past participants—475 were in high school at the time they took the survey and 314 were in college. Clearly, the DNALC's workshops cater to a clientele of interested and able students. Majorities of secondary school (62%) and college (82%) respondents said they had been very interested in science in high school. Virtually all of the students in both groups (97%) said they maintained "A" or "B" averages in their high school science courses.

The DNALC has apparently done a good job of building a clientele for its summer camps. Majorities of both groups rated all seven of the DNALC summer courses as very good or excellent. While the average college respondent had attended two DNALC camps during his/her precollege years, current secondary school students had averaged three camps (with three quarters of respondents still having one or more years until graduation).

Participation in DNALC camps resulted in a number of beneficial effects for the high school and college respondents. Majorities had discussed topics or issues from a workshop with their families (72–75%) and friends (55–59%). Majorities in both groups said that participation in DNALC workshops had increased their understanding of science stories in the media, increased their general interest in science, improved their confidence in science classes, and encouraged them to take more science

offerings. Participation in DNALC workshops strongly influenced 46% of college students to consider majoring in science.

Most importantly, the DNALC experience appears to be part of an education that encourages students to value science and technology and to support scientific research. Note the similar proportions of high school and college respondents who agree with the following statements:

	Percent	
Agreeing	High School	College
Science and technology will open up more opportunities for my generation.	93	94
Science and technology are making our lives healthier, easier, and more comfortable.	92	93
Many scientists want to work on things that will make life better for the average person.	87	88
The benefits of biotechnology outweigh the risks.	71	73
The United States government spends too much money on scientific research.	16	12

The respondents discriminated in similar ways among a number of different applications of gene technology—favoring most, but drawing the line at the selection of physical traits.

Favoring	Percent	
Using genetic engineering to produce essential pharmaceuticals.	88	94
Using genetic engineering to treat diseases in adults.	88	94
Using genetic engineering to treat diseases in the embryo.	79	82
Using genetic engineering to produce agricultural crops.	67	72
Using genetic engineering to clone animals, such as sheep, to make drugs and vaccines.	61	68
Using embryonic stem cells in research.	63	64
Using genetic engineering to select for physical traits in the embryo.	21	17

Complete survey results can be viewed at www.dnalc.org/studentsurveys/.

Saturday DNA!

From the DNALC's wealth of offerings, the ongoing, popular *Saturday DNA!* program has emerged to provide an in-depth view of our genetic book of life and science education to the general public. Using catchy topics such as "CSI: Learning Center" and "Of Lice and Men" as a framework, *Saturday DNA!* allows children, teens, and adults to conduct hands-on DNA experiments and learn about the latest developments in the biological sciences.

Each 2-hour program is designed to be a thorough and exciting exploration of genetics, genes, and DNA through the use of innovative, intriguing topics. The DNALC staff works to ensure that topics are current and appropriate for two different age groups: groups of students ages 10–13 (with an accompanying chaperone) and groups ages 14–adult (with accompanying chaperone for participants under 15).

Although programs change and rotate each season, topics such as "Poochie Pedigrees" and "The Mystery of Anastasia Romanov" are mainstays. In examining the disappearance of the Romanov ruling family, visitors use a mitochondrial DNA database to determine whether Anna Anderson and Anastasia Romanov are indeed one and the same. Along the way, they collect evidence and use a variety of forensic analyses.

"Poochie Pedigrees" offers the opportunity to extract DNA from your dog's cells and compare it to

In the *Jumping Genes* session of *Saturday DNA!* in October, participants got a close look at the life and work of Nobel Prize recipient Barbara McClintock. Through laboratory and computer activities, they learned about the history of classical genetics, and the world of jumping genes.

the DNA of different dogs and their ancestors. Analysis between the various samples raises—and answers—compelling questions such as What is the origin of man's best friend?, Does Fido have wolf or coyote in his bloodlines?, and Are different breeds of dogs really genetically the same? The related program, "Doggie Diagnosis," works with the older age group to take the information a step further to determine how breeding has affected your pet's ability (or lack thereof) to metabolize antibiotics.

Staff and Interns

During the summer, we bid farewell to three senior staff members who made significant contributions to the development of the DNALC. Danielle Sixsmith, Manager of External Relations, moved to New Jersey with her family and began online graduate study at the University of Phoenix. Danielle joined the DNALC staff in 1999 as a laboratory instructor, teaching at both the middle and high school levels. In 2002, she collaborated with North Shore–Long Island Jewish Health System to bring into operation our satellite facility, DNALC *West*. Laboratory Manager Scott Bronson left for a position as Educational Programs Administrator at Brookhaven National Laboratory. Scott joined the DNALC in 1999, and his prior bench experience with Jacek Skowronski was evident as he simplified PCR for education and developed our successful *DNA Sequencing Service*. Media Producer Bronwyn Terrill left for Cambridge, England to manage public outreach at the prestigious Wellcome Trust Sanger Institute. Arriving at the DNALC in 2001, she proved herself to be a world-class science communicator—first producing the 1500-square-foot exhibit "The Genes We Share" and then leading production of the Internet sites *DNA Interactive* and *Inside Cancer*.

Junior staff members Kim Kessler and Mike O'Brien went in different directions in education. Kim enrolled in the genetic counseling program at Northwestern University, while Mike joined the New York City Teaching Fellows to prepare for a teaching position at the High School for the Humanities. Senior Development Officer Erin Wahlgren, who had so effectively run the DNALC golf tournament and annual fund drive, left for a position at the Marfan Foundation.

The major staff changeover brought a raft of new faces and ideas to the DNALC. During the year, we welcomed two Ph.D.-level Laboratory Managers—Tom Bubulya and Craig Hinkley. Although Craig came from University of Michigan, he had formerly shared a bench with Tom as a postdoctoral fellow in the lab of Winship Herr. Tracy Behar, Jeanette Collette, and Lauren Weidler took positions as Laboratory Instructors. Tracy grew up on Long Island and recently graduated from SUNY College at Old Westbury with a degree in Secondary Education in Biology. Jeanette, who also grew up on Long Island, has relocated from Buffalo where she attended college and started a family. While working on her degree in human biology from SUNY Albany, Lauren worked as a library aide in the CSHL Library. Local resident Karen Orzel, who has a background in marketing, took over management of the Corporate Advisory Board, annual golf tournament, and annual fund.

Other new additions to the DNALC "family" came in July when middle school educator Elna Carrasco was married to Michael Gottlieb. Media designer Chun-Hua Yang was married to Shiuh-Jyh Lin in October. Middle school educator Amanda McBrien and her husband, Michael, welcomed their third son, Robert Michael, in November.

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. As part of our new NSF grant, David Wagman used RNA interference to silence a gene in *C. elegans*—resulting in visible "blisters" in the worm's outer cuticle. Daisy Choi injected the human cancer gene, *p53*, into *C. elegans* to study tumor suppression. While working in the lab of Dr. Rob Martienssen, Kimberly Izzo used fluorescent in situ hybridization (FISH) to study centromere function in the model plant *Arabidopsis thaliana*.

Our sequencing service continues to grow with the help of college interns Alina Duvall (Hofstra University) and Andrew Diller (Dowling College). To process the growing number of requests, we now collaborate with Dr. Dick McCombie's group at the Woodbury Genome Center. The high-throughput capillary sequencing available at Woodbury has greatly reduced the turnaround time for completing sequencing requests.

Joining the intern program in 2004 were Andrew Langer (John H. Glen High School, Elwood), Elena Meliuso (Oyster Bay High School), Tama Mizuno (Northport High School), and Benjamin Tully (Nassau Community College). Several interns returned from college to assist with summer workshops: Lara Abramowitz (University of Rochester), Yan Huang (Notre Dame 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: Benjamin Blond (Syosset High School) is studying biology at Amherst College; Bryn Donovan (Freeport High School) is studying wildlife conservation at the University of Delaware; Alexander Hogg (Friends Academy) began a 5-year biology/fine arts program at Brown University; and Michelle Louie (Kings Park High School) began a 7-year biology/M.D. program at George Washington University.

David A. Micklos
Executive Director

2004 Workshops, Meetings, and Collaborations

January 6	Site visit by Alastair Balls and Linda Conlon, Life Science Centre, Newcastle, United Kingdom
January 8–12	Site visit by Cheong Kam Khow, Singapore Science Center
January 10	*Saturday DNA!* Seminar, DNALC
January 24	*Saturday DNA!* Seminar, DNALC
January 26	*Inside Cancer* interview, Ken Culver, Executive Director, Early Clinical Development, Novartis Oncology, East Hanover, New Jersey
February 7	*Saturday DNA!* Seminar, DNALC
February 11	Site visit and museum tour by members of the Association of Suffolk Superintendents for Education Technology
January 12	Site visit by Alan Fleischman, Senior Vice President, New York Academy of Medicine, New York
	Site visit by Don McGranaghan, Artistic Resources, LLC
February 21	*Saturday DNA!* Seminar, DNALC
February 24	West Side School Lecture
March 6	*Saturday DNA!* Seminar, DNALC
March 10	Site visit by Cynthia Joyce, Executive Director, and Loren Eng, President, Spinal Muscular Atrophy Foundation, New York
March 15	West Side School Lecture
March 19	*Saturday DNA!* Seminar, DNALC
March 20	*Saturday DNA!* Seminar, DNALC
March 23	Site visit by Jeremiah Barondess, President, and Patricia Volland, Senior Vice President, The New York Academy of Medicine, New York, and Lawrence Scherr, Dean and Chief Academic Officer, North Shore–Long Island Jewish Health System, Great Neck, New York
	Site visit by Caroline Lieber, Director, Human Genetics Program, Sarah Lawrence College, Bronxville, New York
March 26	Site visit and museum tour of DNALC for representatives of "Best of New York" publication
March 30	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 3	*Saturday DNA!* Seminar, DNALC
April 17	*Saturday DNA!* Seminar, DNALC
April 20	*Great Moments in DNA Science* Honors Students Seminar, CSHL
	Site visit by Ed Rover, President, and Barbara Gill, Vice President, the Dana Foundation, New York
April 26	Site visit by Jon Fry, President; Bruce Pipes, Provost; Fred Owens, Associate Dean of Faculty and Professor of Psychology; and Kathy Triman, Professor of Biology, Franklin & Marshall College, Lancaster, Pennsylvania
	Presentation at Garden City Public Library, Advocacy for Gifted and Talented Education in New York State, Inc.
April 27	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 29	Site visit by Bryan Sykes, Professor of Human Genetics, University of Oxford, United Kingdom
May 14	Site visit by Kidgie Williams, diplomats, and family members of UN representatives, Hospitality Committee for United Nations Delegations, Inc., New York
	Site visit by John Monaghan and Alan Minz, Canadian Consulate General's Office, and Jay Amer, President, Ontario East Economic Development Corporation, Canada
May 15	*Saturday DNA!* Seminar, DNALC
May 20	Museum tour and lab participation by Estee Lauder European Beauty Editors
May 31–June 4	Teacher-training workshops, National Institute of Education and Singapore Science Centre, Singapore
June 9	Site visit by Caroline Lieber, Sarah Lawrence College, Bronxville, New York
June 11	Presentation of *Award of Appreciation* from Kings Park Central School District, New York
June 12	*Saturday DNA!* Seminar, DNALC
June 15	Site visit by Karl Kuchler, University of Vienna, Austria
June 21–25	NSF *Plant Molecular Genetics and Genomics Workshop*, Austin Community College, Texas
June 22	Site visit by Jacinta Duncan, Gene Technology Access Centre, Melbourne, Australia
June 23	*Inside Cancer* interview, William Nelson, The Sidney Kimmel Comprehensive Cancer Center, and Jennifer E. Axilbund, Genetic Counselor, Cancer Risk Assessment Program, The Johns Hopkins Hospital, Baltimore, Maryland
June 24	*Inside Cancer* interview, Louis M. Staudt and Kenneth H. Kraemer, National Cancer Institute, Bethesda, Maryland
June 28–July 2	*Fun With DNA* Workshop, DNALC

	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
July 5–9	*Fun With DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNALC
	Genomic Biology and PCR Workshop, DNALC
July 6	*Inside Cancer* interview, John Condeelis, Albert Einstein College of Medicine, New York
July 7	*Inside Cancer* interview, Glorian Sorensen, Harvard School of Public Health, Boston, Massachusetts
July 8	*Inside Cancer* interviews, Nancy Mueller and Graham Colditz, Department of Epidemiology, and Walter Willett, Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts
July 12–16	*Bioinformatics in the Classroom* Workshop, Union College, Schenectady, New York
	World of Enzymes Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNALC
July 12–30	Pfizer *Leadership Institute in Human and Plant Genomics*, DNALC
	NSF Faculty Fellowship, *Plant and Rice Genomics*: Javier Gonzalez-Ramos, Adriana Robbins, Olga Kopp, Elise Unice, Paul DeLaRosa
	NSF Faculty Fellowship, *Gramene Bioinformatics*, Cesar Gutierrez
	Teacher training for *Singapore Ministry of Education* collaborators, Daniel Chua Wei Sheong and Yin Leng Tan, and Eugene Wambeck of the Singapore Science Center
July 18–23	*Fun With DNA* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
July 26–30	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
July 27	Site visit by Arthur Spiro, CSHL Trustee and DNALC Committee Chairman; Russ Hotzler, John Mogulescu, Nicholas Michelli, Gillian Small, and Tracy Meade, The City University of New York; Sy Fliegel, Cole Glenn, and Reggie Landeau, Center for Educational Innovation–Public Education Association, New York; Edward Travaglianti, Commerce Bank Long Island, New York; and Lawrence Scherr, North Shore–Long Island Jewish Health System, Great Neck, New York
July 30	Site visit by members of the American Association of University Women, Washington, D.C.
August 2–6	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	DNA Science Workshop, DNALC
	NSF *Plant Molecular Genetics and Genomics Workshop*, Clemson University, South Carolina
August 5–6	Site visit by Beverly Matthews, Harvard University, Cambridge, Massachusetts
August 9–13	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Green Genes Workshop, DNALC
	NSF *Plant Molecular Genetics and Genomics Workshop*, University of Wisconsin-Madison/Madison Area Technical College, Madison, Wisconsin
August 12	Site visit by members of the New York Center for Teacher Development
August 16–20	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
	Genomic Biology and PCR Workshop, DNALC
August 17	*Continuing Medical Education* meeting, Lawrence Sherr, Robin Wittenstein, and Irene Leff, North Shore–Long Island Jewish Health System, Great Neck, New York; Caroline Leiber and Jamie Speer, Sarah Lawrence College, Bronxville, New York
August 19	Site visit by Daniel G. Kaufmann, Joan Japha, Chandrika Kulatilleke, Seymour Schulman, Edward Tucker, Mary Jean Holland, and Valerie Schawaroch, faculty members, Department of Natural Sciences, Baruch College, New York
August 20	*Inside Cancer* interview, Charles Sawyers, University of California, Los Angeles
August 23–27	*Fun With DNA* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
Aug. 30–Sept. 3	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Genetic Horizons Workshop, DNALC
September 1	Site visit by Jean Caron, Abby Demars, and Holly Harrick, DNA EpiCenter, New London, Connecticut

September 6–12	Tour and lab instruction for members of the Marino Golinelli Foundation, coinstructed by Marina and Marcello Siniscalco of the Marino Golinelli Foundation, Bologna, Italy
September 16	Site visit by John Phelan, Trustee and Managing Partner, MSD Capital, New York
September 25	*Saturday DNA!* Seminar, DNALC
September 30	Site visit to DNA EpiCenter, New London, Connecticut
October 15	Site visit by students from Zhejiang University, Hangzhou, China
October 16	*Saturday DNA!* Seminar, DNALC
October 21	Site visit by Jennifer Heim, Richard Osborn, and Cindy Encarnacion, St. Louis Science Center, St. Louis, Missouri
October 26	Site visit by Kevin Ward, George Stranahan, and Andrei Rukinstein, Aspen Institute for Physics, Aspen, Colorado
October 27	West Side School Lecture
November 5	Site visit by Patricia Volland and Julia Rankin, New York Academy of Medicine, Board of Education, New York
November 8	Site visit by Glenda Leslie, School of Education at Murdoch University, Australia
November 9	American Association of University Women reception, Washington, D.C.
November 9–19	Teacher training for *Singapore Ministry of Education* collaborators, Tan Hong Kim, Christine Sim, Chan Ter Yue, and Margaret Wong
November 13	*Saturday DNA!* Seminar, DNALC
November 15–17	Site visit by Abby Demars and Holly Harrick, DNA EpiCenter, New London, Connecticut
November 17	West Side School Lecture
	Site visit by Jacqueline Dorrance, Executive Director, Beckman Foundation, Irvine, California
November 18	Site visit by Caroline Fostel, Astoria Federal Savings, New York
November 20	*Saturday DNA!* Seminar, DNALC
Nov. 22–Dec. 3	Teacher-training workshops, National Institute of Education and Singapore Science Centre, Singapore
Nov. 29–Dec. 10	Training for *Singapore Ministry of Education* collaborators, Mohan Krishnamoorthy, Chng Swee How James, Andrea Guo Li Ling, Regina Lim, Yap Lih Min Samuel, Goh Su Fen, Lim Lay Khim, Sixtus Goh Wee Leng, and Nora Teo
December 2	Site visit by staff from DNA EpiCenter, New London, Connecticut

Sites of Major Faculty Workshops 1985–2004

Key: High School **College** *Middle 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**
	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
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	2004
	Bronx High School of Science	1987
	Columbia University, New York	**1993**
	Cold Spring Harbor High School	1985, 1987
	DeWitt Middle School, Ithaca	*1991, 1993*

	DNA Learning Center	1988–1995, 2001–2004
	DNA Learning Center	**1990, 1992, 1995, 2000**
	DNA Learning Center	*1990–1992*
	Fostertown School, Newburgh	*1991*
	Huntington High School	1986
	Irvington High School	1986
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mt. Sinai School of Medicine, New York	1997
	Orchard Park Junior High School	*1991*
	Plainview-Old Bethpage Middle School	*1991*
	The Rockefeller University, New York	2003
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Stuyvesant High School, New York	1998–1999
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Trudeau Institute, Lake Saranac	2001
	Union College, Schenectady	2004
	U.S. Military Academy, West Point	**1996**
	Wheatley School, Old Westbury	1985
NORTH CAROLINA	CIIT 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
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	2001–2004
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995
	Uppsala University, Uppsala	2004

COLD SPRING HARBOR LABORATORY PRESS

2004 PUBLICATIONS

SERIALS

Genes & Development, Vol. 18, 1–3164 (www.genesdev.org)

Genome Research, Vol. 14, 1–2536 (www.genome.org)

Learning and Memory, Vol. 11, 1–804 (www.learnmem.org)

Protein Science, Vol. 13, 1–3382 (www.proteinscience.org)

RNA, Vol. 10, 1–1986 (www.rnajournal.org)

Cold Spring Harbor Symposia in Quantitative Biology, Vol. 68: *The Genome of* Homo sapiens (www.cshl-symposium.org)

LABORATORY MANUALS

Live Cell Imaging: A Laboratory Manual, David Spector and Robert Goldman (eds.), "Live Cell Imaging" (DVD)

Imaging in Neuroscience and Development: A Laboratory Manual, Rafael Yuste and Arthur Konnerth (eds.)

HANDBOOKS

Fly Pushing, 2nd edition, Ralph J. Greenspan

An Illustrated Chinese-English Guide for Biomedical Scientists, James Samet, Weidong Wu, Yuh-Chin T. Huang, and Xinchao Wang (www.chinese-english.cshl.org)

At the Bench, updated edition, Kathy Barker

Mouse Phenotypes: A Handbook of Mutation Analysis, Virginia E. Papaioannou and Richard R. Behringer

Drosophila: *A Laboratory Handbook*, Michael Ashburner, Scott Hawley, and Kent Golic

TEXTBOOKS

A Genetic Switch (3rd ed.), Phage Lambda Revisited, Mark Ptashne

Bioinformatics: Sequence and Genome Analysis (2nd ed.), David Mount (www.bioinformaticsonline.org)

MONOGRAPHS

Cell Growth: Control of Cell Size, Michael N. Hall, George Thomas, and Martin Raff (eds.)

Gastrulation: From Cells to Embryo, Claudio Stern (ed.) (www.gastrulation.org)

GENERAL INTEREST

Is It in Your Genes? The Influence of Genes on Common Disorders and Diseases That Affect You and Your Family, Philip R. Reilly (www.is-it-in-your-genes.org)

Mendel's Legacy: The Origin of Classical Genetics, Elof Axel Carlson

The Writing Life of James D. Watson, Errol C. Friedberg

OTHER

Banbury Center Annual Report 2003

CSHL Annual Report 2003

CSHL Annual Report 2003: Yearbook Edition

Watson School of Biological Sciences Annual Report 2003

WEB SITES

www.BioSupplyNet.com, online directory of scientific supplies and suppliers

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, with a staff of 49, offices on the Woodbury campus, and marketing operations based in San Diego, California and Oxford, United Kingdom. In 2004, the Press had income of $9.5 million and made a total contribution to the Laboratory's economy of more than $1.2 million.

Our research journals, books, and manuals assist the continuing professional education of working scientists and graduate students, our textbooks 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.

Journals

Two thirds of Press income now comes from journal publishing. In 2004, against a downward industry trend, institutional subscriptions to our five journals increased by 4%. Countless numbers of individual readers online downloaded more than 110,000 articles each month in countries around the world. Scientists in the poorest countries are able to access these articles free of charge. More than 12,000 journal pages were published, an increase of over 6%, containing 1200 new articles. Many of these were highlighted in the world's major news media, including *The New York Times*, *The Wall Street Journal*, and the BBC News. All five journals had impact factors that were similar to or higher than last year's, and *Genes & Development* remained one of the ten most highly cited of all primary research journals in science.

Both *RNA* and *Protein Science*, journals published under contract for the RNA and Protein Societies, respectively, continued to reach new levels of institutional subscriptions. Our own journal *Learning & Memory* received a record number of manuscript submissions and, as a result, began publishing monthly issues online and bimonthly issues in print.

Throughout the year, the talk among journal publishers was of an alternative to subscriptions as a business model. "Open access" publishing is paid for by authors, rather than readers, and research papers are made available free of charge online immediately on publication. The model is new and unproven as a means of sustaining an important journal without grant support or private underwriting, but to assess support, *Genome Research* has introduced an immediate free-access option for individual papers. The archives of our journals have been freely accessible online for over 5 years, and this year, the interval between publication and the free release of papers published in *Genes & Development* and *Genome Research* was reduced from 1 year to 6 months. All three Cold Spring Harbor journals are also freely accessible through PubMedCentral.

Some organizations that fund research have begun to voice opinions on the access policies of journals that publish that research. In the United States, the largest supporter of biomedical research is the National Institutes of Health (NIH). Its Director, Dr. Elias Zerhouni, presented a proposal for a database that would provide free access to papers describing NIH-funded research within a very short time of publication. In the intense debate among publishers and scientists that followed, the public benefit of such access was weighed against the potential harm to the financial viability of many subscription-based journals, particularly those published by not-for-profit organizations that use that income to support other activities. I was one of a group of publishers who met with Dr. Zerhouni to discuss and clarify the details and intent of his proposal. The final version of the NIH policy requires the deposition of final manuscripts, rather than published papers, within a period of less than 1 year after acceptance that the authors themselves must decide. The scene is set for conflict between society publishers and their members.

Books and Electronic Media

Laboratory protocols—instructions for executing research techniques—have been the core of our book publishing program for more than 20 years in the form of laboratory manuals widely regarded as the best in their fields. In 2004, a partnership was struck with the new journal *Nature Methods*, in which Press protocols reached a large international audience through two new channels: as a section in each monthly issue of the journal and through access by the journal's institutional subscribers to the online version of our best-selling manual *Molecular Cloning*.

There were two manuals among the 15 new books published in 2004, a list (see p. 388) that included monographs, handbooks, textbooks, general interest and history books, as well as the Annual Symposium volume, this year devoted to the human genome. The Symposium series, first published in 1933, is one of the oldest and most prestigious in biology. For the first time, individual chapters of the proceedings were published online in advance of the printed volume on a Web site that gave viewers a taste of what was happening as the conference actually took place. Combinations of traditional print and digital media were further explored in the manual *Live Cell Imaging*, which includes a DVD showing cells in action, and the monograph *Gastrulation*, whose Web site contains movies of this important event in embryonic development.

Such online enhancements are particularly necessary for textbooks that compete for adoption in crowded markets. Our book *Bioinformatics: Sequence and Genome Analysis*, by David Mount, captured this emergent field on first publication in 2001 and regained its lead with this year's second, extensively revised edition. And the newly released *Molecular Biology of the Gene*, the first new edition in 17 years, also made a strong showing, through the sales expertise of our co-publisher Benjamin Cummings.

Our list of professional handbooks grew still further with the addition of new editions of *Drosophila*, *At The Bench*, and *Fly Pushing*, as well as a new guide to mutation analysis in mice and a reference book for students whose first language is Chinese. Our other books included an ambitious attempt by Philip Reilly to provide a guide for general readers to the genetics of many complex human diseases. In addition, in *The Writing Life of James D. Watson*, Errol Friedberg reminded us that the chancellor of this Laboratory, world famous as a scientist, also has an extraordinary variety of accomplishments as an author.

It was gratifying to note the frequency and warmth of the reviews of our 2003 and 2004 books. One reviewer remarked that "Cold Spring Harbor books are usually exceptional"—nicely summarizing our hopes for all our titles. The biography of geneticist George Beadle, *The Uncommon Farmer*, by Paul Berg and Maxine Singer, was particularly acclaimed and named one of *Choice* magazine's top books of the year. There was also widespread praise for Elof Carlsson's *Mendel's Legacy*, an exploration of the origins of classical genetics. Recognition of the quality of our program has led to an ever-increasing number of foreign translations, and in 2004, 15 such agreements were signed for Chinese, Japanese, Korean, and Italian editions.

In South Africa, our 2001 book for teenagers about HIV/AIDS received intense scrutiny from a diverse group of teachers, students, and community workers in rural and urban areas as we worked to tailor a second edition of the book to evolving public attitudes toward the epidemic and the availability of treatment options. Reflecting these changes, *Staying Alive* has a new, bolder title, *You, Me and HIV*, but the entirely revised content retains the unique, engaging style of its creators Fran Balkwill and Mic Rolph. With the assistance of a grant from the Gates Foundation, 50,000 copies in English and 20,000 copies in Zulu and Afrikaans will be printed for free distribution to schools and health organizations within South Africa, along with a guide to teaching from the book.

In a generally busy year, 25 contracts for new books were signed, including two exciting and innovative textbooks. In addition, the online directory of laboratory supplies and suppliers, BioSupplyNet.com, which we reacquired in 2003, was relaunched with a much improved design and new, attractive functions that generated substantial advertising revenue. These and other initiatives help pave the way for future growth. Other innovations increased efficiency, allowing us to do more with current staff and resources. Significant savings were gained through outsourcing composition and establishing relation-

ships with printers abroad, and by further development of electronic data interchange for managing thousands of book orders and journal subscriptions.

Catalogs and exhibits remain important to our marketing strategies, but more extensive use is being made of online book and journal promotion. Sales through our own Web site continue to grow, but an ever-larger proportion of our books, as much as 60%, now reach our scientist customers through resellers, particularly Amazon.com, with its extraordinary market penetration. Our roster of wholesale customers, international agents, and distributors continues to expand. Scion Publishing, the United Kingdom company trading as CSHL Press Europe, had an encouraging first year, meeting sales projections. It will shortly begin publishing its own books that the Press will distribute in the United States. This arrangement is made possible by our professionally staffed customer service and warehouse operations, which in 2004 processed orders for and shipped 60,000 books and managed inventory to over 99% accuracy. Another recently founded publishing house, Roberts Publishing, has also contracted with us for fulfillment services in the United States.

After the financial challenges of the previous year, I am glad to be able to conclude that 2004 was in contrast a year of growth, new initiatives, and increased confidence for Cold Spring Harbor Laboratory Press as we continued to support the Laboratory's educational mission with first-class publications for scientists, students, and the public worldwide.

Staff

The staff members of the Press as of December 2004 are listed elsewhere in this volume. They bring a cheerful commitment to their work that makes them a pleasure to be with.

In 2004, we welcomed five new colleagues: Laureen Connell, Hillary Sussman, Maria Smit, Kathy Bubbeo, and Mary Mulligan. We also said goodbye to Laurie Goodman, Jill Manero, Joan Pepperman, and Melissa Frey.

In recent years, the activities of the Press have grown in diversity and complexity. The success of this growth is due to a talented and motivated group of departmental managers: Jan Argentine, Ingrid Benirschke, Kathy Cirone, Kathryn Fitzpatrick, Nancy Hodson, Geraldine Jaitin, Bill Keen, Guy Keyes, Marcie Siconolfi, Linda Sussman, and Denise Weiss. They have all taken on new responsibilities with little or no extra help or reward. The Press owes much to them and also to the energy and flair of the editors of our journals, Terri Grodzicker at *Genes & Development*, and Laurie Goodman and her successor Hillary Sussman at *Genome Research*. It is also a pleasure to acknowledge the indispensable Elizabeth Powers who carries out all manner of essential activities in our office with quiet grace.

John R. Inglis
Executive Director

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2004
With comparative financial information as of December 31, 2003

		2004	2003
Assets:			
Cash and cash equivalents	$	40,744,417	28,971,185
Accounts receivable:			
Publications		1,308,718	1,257,221
Other		1,179,604	1,332,279
Grants receivable		6,975,455	7,068,387
Contributions receivable, net		12,492,687	11,452,991
Publications inventory		2,945,099	2,206,640
Prepaid expenses and other assets		1,605,707	1,566,991
Investments		211,294,366	198,696,165
Investment in employee residences		4,830,425	4,774,834
Restricted use assets		2,904,923	2,709,576
Land, buildings, and equipment, net		110,757,023	109,253,408
Total assets	$	397,038,424	369,289,677
Liabilities and Net Assets:			
Liabilities:			
Accounts payable and accrued expenses	$	7,429,657	5,975,281
Notes payable		107,775	140,958
Deferred revenue		3,878,738	4,067,960
Bonds payable		45,200,000	45,200,000
Total liabilities		56,616,170	55,384,199
Net assets:			
Unrestricted		184,397,408	178,415,767
Temporarily restricted		25,419,056	14,223,309
Permanently restricted		130,605,790	121,266,402
Total net assets		340,422,254	313,905,478
Total liabilities and net assets	$	397,038,424	369,289,677

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2004
With summarized financial information for the year ended December 31, 2003

	Unrestricted	Temporarily Restricted	Permanently Restricted	2004 Total	2003 Total
Revenue and other support:					
Public support (contributions and nongovernment grant awards)	$ 10,582,015	17,086,618	1,159,665	28,828,298	29,792,003
Government grant awards	29,451,302	–	–	29,451,302	26,878,120
Indirect cost allowances	19,089,893	–	–	19,089,893	18,000,004
Program fees	3,644,734	–	–	3,644,734	3,134,443
Publications sales	9,743,639	–	–	9,743,639	10,052,963
Dining services	3,183,440	–	–	3,183,440	2,927,668
Rooms and apartments	2,632,671	–	–	2,632,671	2,347,090
Royalty and licensing fees	926,913	–	–	926,913	869,826
Investment income (interest and dividends)	3,134,480	–	–	3,134,480	4,141,495
Miscellaneous	514,559	–	–	514,559	616,755
Total revenue	82,903,646	17,086,618	1,159,665	101,149,929	98,760,367
Net assets released from restrictions	5,890,871	(5,890,871)	–	–	–
Total revenue and other support	88,794,517	11,195,747	1,159,665	101,149,929	98,760,367
Expenses:					
Research	52,049,866	–	–	52,049,866	45,619,807
Educational programs	13,238,766	–	–	13,238,766	12,917,185
Publications	9,380,833	–	–	9,380,833	10,631,334
Banbury Center conferences	1,159,499	–	–	1,159,499	1,136,159
Dolan DNA Learning Center programs	1,611,325	–	–	1,611,325	1,809,174
Watson School of Biological Sciences programs	2,263,518	–	–	2,263,518	2,029,275
General and administrative	12,508,768	–	–	12,508,768	11,976,718
Dining services	4,236,535	–	–	4,236,535	4,262,515
Total expenses	96,449,110	–	–	96,449,110	90,382,167
(Deficiency) excess of revenue and other support over expenses	(7,654,593)	11,195,747	1,159,665	4,700,819	8,378,200
Other changes in net assets:					
Net appreciation in fair value of investments	13,636,234	–	8,179,723	21,815,957	38,128,865
Increase in net assets	5,981,641	11,195,747	9,339,388	26,516,776	46,507,065
Net assets at beginning of year	178,415,767	14,223,309	121,266,402	313,905,478	267,398,413
Net assets at end of year	$ 184,397,408	25,419,056	130,605,790	340,422,254	313,905,478

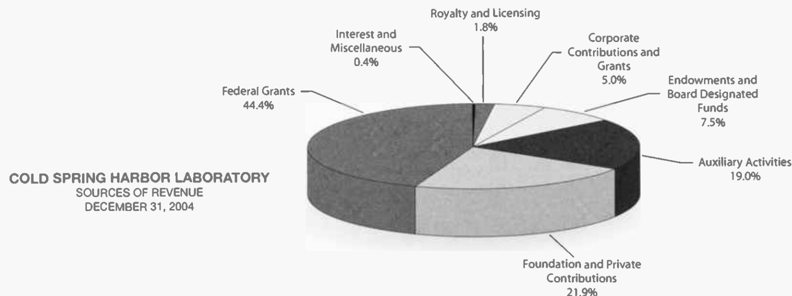
CONSOLIDATED STATEMENTS OF CASH FLOWS
Year ended December 31, 2004
With comparative financial information for the year ended December 31, 2003

	2004	2003
Cash flows from operating activities:		
Increase in net assets	$ 26,516,776	46,507,065
Adjustments to reconcile increase in net assets to net cash used in operating activities:		
Depreciation and amortization	5,769,622	5,458,719
Net appreciation in fair value of investments	(21,746,064)	(37,868,190)
Contributions restricted for long-term investment	(13,812,552)	(13,460,462)
Changes in assets and liabilities:		
Decrease (increase) in accounts receivable	101,178	(459,802)
Decrease (increase) in grants receivable	92,932	(1,912,888)
Decrease (increase) in contributions receivable	79,666	(875,375)
(Increase) decrease in publications inventory	(738,459)	7,859
(Increase) decrease in prepaid expenses and other assets	(38,716)	63,375
(Increase) decrease in restricted use assets	(195,347)	355,653
Increase in accounts payable and accrued expenses	1,454,376	802,963
(Decrease) increase in deferred revenue	(189,222)	419,507
Net cash used in operating activities	(2,705,810)	(961,576)
Cash flows from investing activities:		
Capital expenditures	(7,273,237)	(6,296,049)
Proceeds from sales and maturities of investments	113,998,317	135,393,757
Purchases of investments	(104,850,454)	(122,075,553)
Net change in investment in employee residences	(55,591)	(427,207)
Net cash provided by investing activities	1,819,035	6,594,948
Cash flows from financing activities:		
Permanently restricted contributions	1,159,665	4,822,932
Contributions restricted for investment in land, buildings, and equipment	12,652,887	8,637,530
Increase in contributions receivable	(1,119,362)	(6,092,438)
Repayment of notes payable	(33,183)	(29,161)
Net cash provided by financing activities	12,660,007	7,338,863
Net increase in cash and cash equivalents	11,773,232	12,972,235
Cash and cash equivalents at beginning of year	28,971,185	15,998,950
Cash and cash equivalents at end of year	$ 40,744,417	28,971,185
Supplemental disclosures:		
Interest paid	$ 780,758	1,014,956

COMPARATIVE OPERATING HISTORY 2000–2004
(Dollars in Thousands)

	2000	2001	2002	2003	2004
Revenue:					
Main Lab:					
Grants and contracts	$ 30,345	34,716	37,872	41,749	48,208
Indirect cost allowances	12,718	14,134	14,987	17,869	18,910
Other	10,618	12,528	10,918	10,524	11,611
CSHL Press	8,684	9,941	9,051	10,053	9,744
Banbury Center	1,856	1,666	1,763	1,729	1,779
Dolan DNA Learning Center	1,471	1,878	2,978	2,564	2,314
Watson School of Biological Sciences	682	927	1,496	1,769	1,889
Total revenue	66,374	75,790	79,065	86,257	94,455
Expenses:					
Main Lab:					
Research and training	30,345	34,716	37,872	41,749	48,208
Operation and maintenance of plant	6,589	7,027	8,661	8,702	8,606
General and administrative	6,162	6,492	6,395	7,507	7,836
Other	7,075	9,505	8,550	8,959	9,410
CSHL Press	8,186	9,515	8,962	10,234	8,995
Banbury Center	1,702	1,536	1,597	1,616	1,642
Dolan DNA Learning Center	1,362	1,801	2,780	2,257	2,066
Watson School of Biological Sciences	682	927	1,496	1,769	1,880
Total expenses excluding depreciation	62,103	71,519	76,313	82,793	88,643
Excess before depreciation and (designation) release of funds	4,271	4,271	2,752	3,464	5,812
Depreciation	(3,974)	(4,620)	(5,165)	(5,459)	(5,770)
(Designation) release of funds [1]	(297)	349	1,848	–	–
Net operating excess (deficit)	$ –	–	(565)	(1,995)	42

The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor Laboratory prepares operating budgets.
[1]Funds designated to underwrite future direct and indirect expenses of new research programs.



COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
DECEMBER 31, 2004

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 2004.

GRANTS January 1, 2004–December 31, 2004

COLD SPRING HARBOR LABORATORY GRANTS

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2004 Funding**
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Equipment	Dr. Myers	04/01/04 03/31/05	$ 211,975 •
	Dr. Stillman	09/01/04 08/31/05	984,159 •
Program Projects	Dr. Herr	01/01/97 12/31/06	5,041,821
	Dr. Stillman	08/01/87 07/31/05	3,855,008
Research Support	Dr. Brody	07/01/04 04/30/09	304,650 •
	Dr. Chklovskii	07/08/04 06/30/09	304,650 •
	Dr. Cline	12/10/04 11/30/09	484,030 •
	Dr. Cline	03/01/98 03/31/06	452,805
	Dr. Dubnau	09/15/04 06/30/09	349,800 •
	Dr. Hamaguchi	04/01/04 03/31/08	346,707 •
	Dr. Hannon	07/01/00 08/31/05	289,690
	Dr. Hernandez	07/01/04 06/30/08	245,413 •
	Dr. Herr	03/01/02 02/28/06	298,800
	Dr. Hirano	07/01/01 06/30/05	275,560
	Dr. Hirano	05/01/96 04/30/08	406,000
	Dr. Huang	08/01/01 06/30/06	415,000
	Dr. Joshua-Tor	05/01/01 03/31/06	373,500
	Dr. Joshua-Tor	02/15/02 01/31/07	373,500
	Dr. Krainer	07/01/03 06/30/07	535,378
	Dr. Krainer	06/01/01 05/31/06	408,400
	Dr. Lazebnik	09/01/03 08/31/05	211,666
	Dr. Lowe	070/1/04 06/30/09	390,832 •
	Dr. Lowe	04/01/04 03/31/09	849,999 •
	Dr. Mainen	09/23/02 08/31/07	370,813
	Dr. Malinow	05/01/92 04/30/05	565,477
	Dr. Malinow	04/01/95 02/29/08	553,671
	Dr. Martienssen	08/01/03 07/31/07	338,584
	Dr. Mittal	04/01/04 03/31/09	312,036 •
	Dr. Muthuswamy	03/01/03 02/28/07	376,211
	Dr. Neuwald	09/30/98 08/31/06	373,500
	Dr. Skowronski	04/01/98 04/30/08	640,187
	Dr. Spector	04/01/90 03/31/07	652,102
	Dr. Spector	09/01/04 08/31/08	363,220 •
	Dr. Stein	09/01/02 06/30/05	617,776
	Dr. Stillman	07/01/91 05/31/08	555,907
	Dr. Svoboda	12/01/03 11/30/08	313,498
	Dr. Svoboda	12/01/02 11/30/07	281,724

*Includes direct and indirect costs.
•New grants awarded in 2004.

	Dr. Svoboda	06/01/03	03/31/08	$ 295,969	
	Dr. Svoboda	03/01/04	02/28/09	169,080	*
	Dr. Tansey	05/01/03	04/30/07	337,365	
	Dr. Tonks	08/01/91	03/31/06	646,533	
	Dr. Tonks	05/01/97	06/30/05	348,600	
	Dr. Tully	03/01/03	02/29/08	380,438	
	Dr. Tully	08/01/03	05/31/07	380,719	
	Dr. Van Aelst	05/01/03	04/30/08	338,756	
	Dr. Wigler	07/15/98	04/30/07	502,725	
	Dr. Xu	08/01/01	07/31/05	252,320	
	Dr. Xu	08/01/03	03/31/07	363,619	
	Dr. Zador	01/23/03	12/31/07	422,500	
	Dr. M. Zhang	09/19/03	08/31/06	584,205	
	Dr. Zhong	07/01/03	06/30/08	346,540	
Fellowships	Dr. Ding	07/01/04	06/30/07	42,976	*
	Dr. Kuhlman	12/01/02	11/30/05	48,928	
	Dr. Lucito	06/01/02	05/31/07	140,867	
	Dr. Stepanyants	07/01/04	06/30/09	114,831	*
	Dr. Zilfou	07/01/04	06/30/05	48,928	*
Course Support	Advanced Immunocytochemistry In Situ Hybridization and Live Cell Imaging	07/01/98	08/31/05	76,766	
	C. elegans	08/01/98	08/01/06	67,152	
	Cancer Workships	01/01/83	03/31/06	308,282	
	Cell and Developmental Biology of *Xenopus*	04/01/93	03/31/09	19,440	
	Computational Genomics Course	06/06/91	08/31/07	46,284	
	CSHL Neurobiology Short-term Training	07/01/01	06/30/06	160,527	
	Genome Informatics	07/01/00	06/30/05	50,728	
	Macromolecular Crystallography	09/01/00	08/31/05	56,773	
	Making And Using DNA Microarrays	07/01/04	04/30/07	55,635	*
	Proteomics	07/01/03	06/30/06	66,792	
Meeting Support	69th Symposium: Epigenetics	05/15/01	04/30/06	5,000	
	69th Symposium: Epigenetics	06/04/04	05/31/05	11,000	*
	Axon Guidance and Neural Plasticity	06/01/00	05/31/05	19,000	
	Cancer Genetics Conference	08/01/00	07/31/05	15,000	
	Cell Cycle	04/01/00	03/31/05	9,000	
	Evolution of Developmental Diversity	04/17/02	03/31/05	13,000	
	Gene Expression and Signaling in the Immune System	04/19/02	03/31/07	2,000	
	Germ Cells	07/01/04	06/30/05	6,000	*
	Molecular Chaperones and the Heat Shock Response	04/01/04	03/31/09	13,000	*
	Mouse Molecular Genetics	07/12/02	06/30/07	17,962	
	Pharmacogenomics	08/10/04	07/31/09	18,683	*
	Translational Control	07/05/02	06/30/07	6,000	
NATIONAL SCIENCE FOUNDATION					
Research Support	Dr. Hannon	09/01/04	02/28/06	200,000	*
	Dr. Jackson	03/01/04	02/28/07	130,000	*
	Dr. Jackson	08/01/02	07/31/05	120,000	
	Dr. Jackson	10/01/01	09/30/06	284,058	
	Dr. McCombie	09/01/03	08/31/06	1,303,762	
	Dr. McCombie	06/15/04	11/30/05	199,949	*
	Dr. Stein	09/01/03	08/31/06	556,369	
	Dr. Stein	12/15/03	11/30/07	1,591,214	
	Dr. Timmermans	07/01/02	06/30/05	123,562	

*Includes direct and indirect costs.
*New grants awarded in 2004.

Grant	Investigator/Program	Start	End	Amount	
	Dr. Timmermans	09/01/03	08/31/06	$ 120,077	
	Dr. Ware	09/01/03	08/31/08	290,586	
	Dr. M. Zhang	09/15/03	08/31/08	65,000	
	Drs. Martienssen/McCombie/Stein/Lucito	09/01/01	08/31/05	519,921	
Course Support	Advanced Bacterial Genetics	07/15/04	06/30/09	145,659	*
	Advanced Techniques in Plant Science	07/01/04	06/30/05	76,875	*
	Cereal Genomics	10/01/04	03/31/06	36,030	*
	Computational Neuroscience Vision	06/15/04	05/31/05	52,000	*
	RCN: The Gramene Community Curation Network 4 Workshops	01/15/03	12/31/05	183,087	
Meeting Support	Computational and Systems Neuroscience (Cosyne)	03/15/04	02/28/05	13,780	*
	Plant Genomes: From Sequence to Phenome	08/01/04	07/31/05	15,187	*
	Evolution of Developmental Diversity	03/01/04	02/28/05	16,763	*
	Molecular Genetics of Bacteria and Phage	04/01/04	03/31/05	5,000	*
UNITED STATES DEPARTMENT OF AGRICULTURE					
Research Support	Dr. Jackson	09/01/03	08/31/06	74,988	
	Dr. Martienssen	08/01/03	07/31/05	96,402	
	Dr. McCombie	02/01/04	01/31/07	201,842	*
	Dr. McCombie	09/15/04	09/14/09	50,000	*
	Dr. Stein	09/22/03	09/14/05	250,000	
	Dr. Stein	09/15/02	09/14/05	783,785	
	Dr. Stein	10/01/02	09/30/05	220,000	
	Dr. Stein	03/16/03	09/30/05	838,016	
UNITED STATES DEPARTMENT OF THE ARMY					
Research Support	Dr. Hannon	04/01/02	03/31/06	732,644	
	Drs. Hannon/Lowe	09/01/02	08/31/05	166,000	
	Dr. Mainen	04/01/04	03/31/06	199,969	*
	Dr. Muthuswamy	07/15/04	07/14/05	126,953	*
	Dr. Wigler	04/01/04	03/31/06	1,784,200	*
	Dr. Zhong	12/30/04	12/29/05	100,000	*
	Dr. Zhong	12/15/04	12/14/08	334,511	*
Fellowships	M. Carmel	05/01/02	04/30/05	22,000	
	T. Haire	10/01/04	09/30/06	29,991	*
	A. Lucs	04/01/03	03/31/06	29,991	
	M. Moore	08/16/04	08/15/06	29,991	*
	S. Nunez	02/27/04	02/28/07	30,000	*
	K. Siddiqui	04/01/03	03/31/06	29,991	
	D. Siolas	07/01/04	06/30/06	30,304	*
	Dr. Du	06/01/02	12/31/04	20,427	
	Dr. Kannanganattu	01/01/01	06/30/04	27,858	
	Dr. P. Smith	04/01/04	03/31/07	108,000	*
	Dr. Wendel	09/01/04	08/31/05	100,000	*
	Dr. Xiang	06/01/03	05/31/06	56,984	
MISCELLANEOUS GRANTS					
Equipment					
NYS Grand Lodge Order Sons of Italy/Breast Cancer Help, Inc.	Dr. Stillman	05/01/04	04/30/05	10,000	*

·Includes direct and indirect costs.
*New grants awarded in 2004.

Research Support

Sponsor	Investigator	Start	End	Amount	
AACR–National Foundation for Cancer Research Professorship in Basic Cancer Research	Dr. Lowe	07/01/04	06/30/05	$ 50,000	*
AKC Canine Health Foundation	Dr. McCombie	07/19/01	07/20/04	33,000	
American Cancer Society	Dr. Wigler	01/01/96	12/31/04	10,000	
American Cancer Society	Dr. Wigler	01/01/01	12/31/05	70,000	
The Michael Scott Barish Human Cancer Grant sponsored by 1 in 9: The Long Island Breast Cancer Action Coalition	Dr. Wigler	01/01/04	12/31/04	70,025	
Breast Cancer Research Foundation	Dr. Wigler	10/01/00	09/30/05	250,000	
Dorothy Rodbell Cohen Foundation for Cancer Research	Dr. Tonks	06/14/04	06/15/05	20,000	*
The Charles A. Dana Foundation, Inc.	Dr. Cline	10/01/03	03/31/05	34,006	
The Charles A. Dana Foundation, Inc.	Dr. Van Aelst	04/01/03	09/30/04	33,773	
Dart Neurogenomics Project	Dr. Tully	10/01/04	12/31/04	200,000	*
DeMatteis Family Foundation	Dr. Powers	10/01/04	09/30/05	268,000	*
ELJI/SUSB Consortium Agreement	Dr. Huang	01/15/04	01/14/07	70,481	*
Find A Cure Today (F.A.C.T.)	Dr. Muthuswamy	07/01/03	02/28/05	39,040	
FSMA	Dr. Krainer	07/15/04	07/14/05	20,000	*
Genentech	Dr. Hannon	01/30/04	01/29/05	1,000,000	*
Irving Hansen Foundation	Dr. Tansey	08/01/04	07/31/05	20,000	*
Ira Hazan	Dr. Enikolopov	12/01/04	11/30/05	200,000	*
Harrison's Heart Foundation	Dr. Hatchwell	12/21/04	06/30/05	30,000	*
Helicon	Dr. Tully	07/01/01	06/30/05	84,000	
Human Frontier of Science Program Organization (HFSPO)	Dr. Spector	06/01/03	05/31/06	112,500	
Human Frontier of Science Program Organization (HFSPO)	Dr. Mitra	06/15/03	06/14/06	96,500	
David H. Koch	Dr. Powers	12/01/04	11/30/05	50,000	*
The Leukemia & Lymphoma Society	Dr. Lowe	10/01/03	09/30/08	1,000,000	
Long Beach Breast Cancer Coalition	Dr. Muthuswamy	07/15/04	07/14/05	2,000	
LI 2 Day Walk to Fight Breast Cancer	Dr. Muthuswamy	08/01/04	07/31/05	5,000	*
LIABC (Long Islanders Against Breast Cancer)	Dr. Wigler	01/01/04	12/31/04	72,720	
The March of Dimes	Dr. Huang	06/01/04	05/31/07	287,409	*
The March of Dimes	Dr. Mills	06/01/04	05/31/05	68,686	*
Carol Marcincuk Ovarian Cancer Research Fund	Dr. Lucito	11/01/04	10/31/05	3,695	*
The G. Harold & Leila Y. Mathers Charitable Trust	Dr. Zador	07/01/03	06/30/06	165,388	
The Elizabeth McFarland Breast Cancer Fund	Dr. Wigler	03/01/04	02/28/05	30,424	*
Merck & Co. Inc	Dr. Hannon	04/16/03	04/15/05	700,000	
Louis Morin Charitable Trust	Dr. Spector	12/01/04	11/30/05	100,000	*
NARSAD	Dr. Mainen	09/15/04	09/14/06	50,000	*
NARSAD	Dr. Enikolopov	09/15/04	09/14/05	50,000	*
NIH/Affymetrix Consortium Agreement	Dr. Stein	08/01/04	05/31/06	90,562	*
NIH/Baylor College of Medicine Consortium Agreement	Dr. Mills	09/01/01	08/31/06	220,787	
NIH/Baylor College of Medicine Consortium Agreement	Dr. Muthuswamy	03/01/04	12/31/04	56,756	*
NIH/Booz Allen Hamilton caBIG Consortium Agreement	Dr. Stein	08/01/04	02/28/05	270,400	*
NIH/Caltech Consortium Agreement	Dr. Svoboda	04/04/03	02/29/08	144,023	
NIH/Columbia University Consortium Agreement	Dr. Lowe	09/30/00	07/31/05	455,447	

*Includes direct and indirect costs.
*New grants awarded in 2004.

NIH/Evanston Northwestern Consortium Agreement	Dr. M. Zhang	07/01/03	06/30/06	$ 133,221	
NIH/Massachusetts Institute of Technology Consortium Agreement	Dr. Stein	09/15/04	08/31/05	492,475	*
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Mittal	04/01/04	03/31/09	132,411	*
NIH/Memorial Sloan Kettering Consortium Agreement	Dr. Van Aelst	07/10/03	06/30/08	326,044	
NIH/University of Virginia Consortium Agreement	Dr. Stein	08/01/02	07/31/05	225,834	
NIH/Washington University Consortium Agreement	Dr. Stein	09/01/03	08/31/08	529,246	
NIH/Washington University Consortium Agreement	Dr. Stein	11/01/03	10/31/06	89,891	
NIH/Weill Cornell Medical Center Consortium Agreement	Dr. Mitra	02/01/04	01/31/06	76,622	*
NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	10/01/04	09/30/09	193,006	*
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	10/01/04	09/30/09	257,830	*
NSF/Rutgers University Consortium Agreement	Drs. Spector/Martienssen/McCombie	10/01/00	09/30/05	368,052	
NSF/University of Arizona Consortium Agreement	Dr. Stein	10/01/03	09/30/07	195,862	
NSF/University of Calfornia, Berkeley Consortium Agreement	Drs. Jackson/Martienssen	10/01/01	09/30/06	284,058	
NSF/University of Georgia Consortium Agreement	Dr. Timmermans	09/01/03	08/31/07	229,849	
NSF/University of Wisconsin Consortium Agreement	Dr. Martienssen	09/01/00	08/31/05	304,223	
NSF/University of Wisconsin Consortium Agreement	Dr. Stein	01/01/04	12/31/07	263,887	*
Olin Family Foundation	Dr. Van Aelst	02/01/04	01/31/05	7,000	*
OSI Pharmaceuticals	Dr. Mittal	10/21/02	10/20/05	450,000	
Ovarian Cancer Research Fund	Dr. Lucito	01/01/04	12/31/04	50,000	*
Pfizer, Inc.	Dr. Hannon	12/01/03	11/30/05	312,000	
Philips Research	Dr. M. Zhang	12/01/04	11/30/05	5,000	*
Redwood Neuroscience Institute	Dr. Brody	01/01/04	12/31/04	35,000	*
Redwood Neuroscience Institute	Dr. Koulakov	01/01/04	12/31/04	35,000	*
SAIC–Frederick, Inc. Research Agreement	Dr. Hannon	10/21/02	12/31/05	507,000	
SAIC–Frederick, Inc. Research Agreement	Dr. M. Zhang	03/08/04	03/07/05	71,541	*
The Seraph Foundation	Dr. Enikolopov	10/01/04	09/30/05	69,000	*
The Simons Foundation	Dr. Wigler	11/01/03	10/31/05	1,967,040	
SMA Foundation	Dr. Krainer	06/01/04	05/31/07	59,333	*
SNP Consortium Ltd	Dr. Stein	02/24/03	12/31/05	360,415	
US Army/New York University Consortium Agreement	Dr. Lucito	09/27/04	09/26/09	119,785	*
USDA/Oregon State University Consortium Agreement	Dr. Martienssen	12/01/04	11/30/07	12,800	*
WALK For Women Breast Cancer Fund	Dr. Muthuswamy	04/01/04	03/31/05	5,500	*

Fellowships

Rita Allen Foundation	Dr. Muthuswamy	08/01/04	07/31/05	50,000	*
Alzheimer's Association	Dr. Boehm	10/01/03	09/30/05	50,000	
Alzheimer's Association	Dr. Ijima	10/01/03	09/30/05	50,000	
Burroughs Wellcome	Dr. Yasuda	01/01/03	12/31/07	60,000	

·Includes direct and indirect costs.
*New grants awarded in 2004.

Burroughs Wellcome	Dr. Zito	09/01/02	07/31/05	$ 58,000
Canadian Institute of Health Research (CIHR)	Dr. Li	08/01/04	07/31/07	18,000 *
Jane Coffin Childs Foundation	Dr. Rivas	07/01/04	06/30/07	42,500 *
Jane Coffin Childs Foundation	Dr. Gillespie	07/01/02	06/30/05	44,500
Damon Runyon Cancer Research Foundation	Dr. Hu	02/16/04	07/31/06	42,500 *
Damon Runyon Cancer Research Foundation	Dr. Z. Zhang	04/01/03	03/31/06	42,500
European Molecular Biology Organization (EMBO) Long-term Fellowship	Dr. De Paola	07/01/04	06/30/05	30,600 *
FRAXA Research Foundation	Dr. Bestman	09/01/03	08/31/05	40,000
Goldring Fellowship	Dr. Stillman	09/01/00	08/31/05	75,000
Human Frontier of Science Program Organization (HFSPO)	Dr. Iijima	04/26/02	04/25/05	42,000
Human Frontier of Science Program Organization (HFSPO)	Dr. Bechamel	04/01/03	03/31/06	31,500
Howard Hughes Medical Institute	Graduate Student Support	09/01/94	08/31/05	57,500
Karp Foundation	Dr. Chklovskii	09/16/04	08/15/05	40,000 *
Klingenstein Foundation	Dr. Chklovskii	07/01/04	06/30/07	50,000 *
The Leukemia & Lymphoma Society	Dr. Buckley	11/01/03	10/31/06	40,000
The Leukemia & Lymphoma Society	Dr. Scott	07/01/03	06/30/06	50,000
The Leukemia & Lymphoma Society	Dr. Tansey	07/01/01	06/30/06	100,000
Leukemia Research Foundation	Dr. Dickins	07/01/03	06/30/05	30,000
Life Sciences Research Foundation/ DOE–Energy Biosciences Research Fellowship	Dr. Goto	06/01/04	05/31/07	50,000 *
Maxfield Foundation	Dr. Lazebnik	12/01/04	11/30/05	5,000 *
McKnight Endowment	Dr. Huang	07/01/04	06/30/07	75,000 *
Minister of Education & Science, Spain	Dr. Encinas	05/01/04	04/30/06	23,150 *
NARSAD	Dr. DiCristo	07/01/04	06/30/06	30,000 *
NARSAD	Dr. Piccini	07/01/03	06/30/05	30,000
NARSAD	Dr. Ruthazer	07/01/04	06/30/06	30,000 *
NYSTAR	Dr. Henry	01/01/03	12/31/04	100,000
Pew Charitable Trust	Dr. Huang	07/01/02	06/30/06	60,055
Alfred P. Sloan Foundation	Dr. Brody	09/16/04	09/15/06	20,000 *
Stony Brook–W. Burghardt Turner Fellowship Summer Funding	M. Juarez	06/01/04	07/31/05	3,000 *
Lauri Strauss Leukemia Foundation	Dr. Scott	01/01/04	12/31/04	15,000 *
Swartz Foundation	Dr. Kepecs	01/01/04	12/31/04	50,000 *
Swartz Foundation	Dr. Machens	01/01/04	12/31/04	50,000 *
Swartz Foundation	A. Nikitchenko	01/01/04	12/31/04	25,000 *
Swartz Foundation	Q. Wen	01/01/04	12/31/04	25,000 *
Swartz Foundation	Dr. Mitra	07/01/04	06/30/05	50,000 *
Helen Hay Whitney Foundation	Dr. He	04/01/04	03/31/07	42,500 *
Helen Hay Whitney Foundation	Dr. Hemann	09/01/03	08/31/06	45,000
Helen Hay Whitney Foundation	Dr. Karpova	07/01/02	06/30/05	46,500

Training Support

Baltimore Family Fund	Undergraduate Research Program	04/01/04	12/31/04	10,000 *
Jephson Education Trust	Undergraduate Research Program	05/01/04	12/31/04	10,000 *
Steamboat Foundation	Undergraduate Research Program	05/01/04	12/31/04	12,000 *

Course Support

Eppley Foundation for Research	Stem Cell	03/01/04	02/28/05	37,500 *
Howard Hughes Medical Institute	Neurobiology	01/01/91	12/31/09	330,000
Klingenstein Foundation	Neurobiology	03/01/02	02/28/05	50,000

†Includes direct and indirect costs.
*New grants awarded in 2004.

Meeting Support

Grantor	Program/Project Title	Start	End	Amount
Abbott Laboratories	Gene Expression and Signaling in the Immune System	01/01/04	12/31/04	$ 10,000 *
Advanced Acoustic Concepts	Conference on Computational and Systems Neuroscience (Cosyne)	01/01/04	12/31/04	1,500 *
Alzheimer's Association	Drug Discovery in Neurodegenerative Diseases	09/01/04	08/31/05	2,000 *
Alzheimer's Association	Molecular Chaperones and the Heat Shock Response	01/01/04	12/31/04	1,000 *
Biogen	Gene Expression and Signaling in the Immune System	01/10/04	12/31/04	1,000 *
Boehringer Ingelheim Pharmaceuticals	Gene Expression and Signaling in the Immune System	01/01/04	12/31/04	500 *
Hoffman La Roche	Gene Expression and Signaling in the Immune System	01/01/04	12/31/04	1,000 *
Ina Institute	Molecular Chaperones and the Heat Shock Response	01/01/04	12/31/04	2,000 *
Lalor Foundation	Conference on Germ Cells	10/01/04	09/30/05	5,000 *
March Of Dimes	Conference on Germ Cells	08/01/04	01/31/05	3,500 *
Merck & Co.	Gene Expression and Signaling in the Immune System	01/01/04	12/31/04	1,000 *
Merck & Co.	Molecular Chaperones and the Heat Shock Response	02/01/00	12/31/04	1,000 *
Miscellaneous	Crystallography	01/01/04	12/31/04	3,750
Pfizer	Gene Expression and Signaling in the Immune System	10/01/03	09/30/04	5,000 *
Qiagen, Inc.	Drug Discovery in Neurodegenerative Diseases Meeting	01/01/04	12/31/04	6,000 *
Redwood Neuroscience Institute	Conference on Computational and Systems Neuroscience (Cosyne)	03/01/04	02/28/05	25,000 *
Sloan-Swartz Center	Theoretical Neurobiology Annual Summer Meeting	07/01/04	06/30/05	44,716 *
Swartz Foundation	Conference on Computational and Systems Neuroscience (Cosyne)	01/01/04	12/31/04	21,200 *

Library Support

Grantor	Program/Project Title	Start	End	Amount
Alfred P. Sloan Foundation	To Develop a Memory Board to Capture the Recent History of Science and Technology Online	11/01/04	10/31/05	45,000 *

·Includes direct and indirect costs.
·New grants awarded in 2004.

DOLAN DNA LEARNING CENTER GRANTS

Grantor	Program/Principal Investigator	Duration of Grant	2004 Funding*
FEDERAL GRANTS			
National Institutes of Health			
ELSI Research Program	*Eugenics Archive* Internet Site	3/98–3/04	$ 25,168
Science Education Partnership Award	*Inside Cancer* Internet Site	1/01–12/04	100,089
National Science Foundation	Plant Molecular Genetics and Genomics	2/03–1/06	186,799
Course, Curriculum, and	RNA Interference	6/04–5/06	58,627
Laboratory Improvement Program	Rice Genome Sequencing Education	9/03–8/05	78,726
	Gramene Genome Annotation Education	12/03–11/06	105,017
NONFEDERAL GRANTS			
Biogen Idec Foundation	*Genetic Origins* Program	2004	$ 50,000
Carolina Biological Supply Company	Research support	2004	75,000
Clemson University	License, training, and development	2004	50,000
DNA EpiCenter	License, training, and development	2004	1,250
William A. Haseltine Foundation	*Genetic Origins* Program	2004	10,000
Howard Hughes Medical Institute	*DNA Interactive*	1/02–6/04	25,369
North Shore–LIJ Health System	DNALC *West* support	2004	50,000
Pfizer Foundation	Leadership Institute	2004	75,000
Pfizer Foundation	DNA EpiCenter support	2004	17,000
Edwin S. Webster Foundation	Unrestricted support	2004	20,000
Roberson Museum and Science Center	License, training, and development	2004	6,250
Singapore Ministry of Education	License, training, and development	2004	177,715

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
East Meadow Union Free School District	1,250
Elwood Union Free School District	1,250
Fordham Preparatory School	2,750
Great Neck Union Free School District	2,500
Green Vale School	1,250
Harborfields Central School District	1,250
Herricks Union Free School District	2,500
Island Trees Union Free School District	2,500
Jericho Union Free School District	1,250
Kings Park Central School District	1,500
Levittown Union Free School District	1,250
Long Beach City School District	$ 1,250
Massapequa Union Free School District	2,500
North Shore Central School District	2,500
North Shore Hebrew Academy High School	2,750
Oceanside Union Free School District	1,250
Oyster Bay–East Norwich Central School District	2,500
Plainedge Union Free School District	1,250
Plainview–Old Bethpage Central School District	1,250
Port Washington Union Free School District	1,250
Syosset Central School District	1,250
West Hempstead Union Free School District	1,250

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
Bay Shore Union Free School District	2,160
Bellmore Union Free School District	2,750
Bellmore–Merrick Central School District	13,075
East Meadow Union Free School District	2,870
East Williston Union Free School District	2,380
Elwood Union Free School District	3,712
Farmingdale Union Free School District	2,450
Friends Academy	3,050
Garden City Union Free School District	6,935
Great Neck Union Free School District	9,050
Green Vale School	1,180
Half Hollow Hills Central School District	5,512
Harborfields Central School District	12,360
Huntington Union Free School District	5,427
Jericho Union Free School District	13,962
Kings Park Central School District	$ 1,595
Lawrence Union Free School District	7,200
Locust Valley–Bayville Central School District	19,180
Mamaroneck Union Free School District	2,800
North Bellmore Union Free School District	2,055
Oyster Bay–East Norwich Central School District	2,107
Port Washington Union Free School District	1,180
Region 3, Queens	1,625
Rockville Center Union Free School District	4,735
Sachem Central School District	1,700
Scarsdale Union Free School District	3,500
South Colonie Central School District	2,490
St. Dominic School	3,500
St. Edward School	1,520
Syosset Central School District	26,200
Three Village Central School District	1,925

*Includes direct and indirect costs.

BANBURY CENTER GRANTS

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2004 Funding*[*]
FEDERAL SUPPORT			
Centers for Disease Control and Prevention (CDC)	Integrating Disparate Data to Stimulate Lymphocyte Function	2004	$ 45,000*
NIH–National Institute of Mental Health (through a grant to University of Illinois)	New Pharmacological Approaches to the Treatment of Fragile X	2004	35,441*
U.S. Department of Homeland Security (through a grant to UMDNJ–New Jersey Medical School	Microbial Forensics	2004	50,000*
NONFEDERAL SUPPORT			
Meeting Support			
Den Haag Foundation	Breast Cancer Research: A Critical Review for Future Strategies	2004	12,500*
Institute for Comparative Genomics	New Insights into Viral Disease from Mathematical Modeling of Biological Systems	2004	42,765*
Albert B. Sabin Vaccine Institute, Inc. (with the support of the Bill & Melinda Gates Foundation)	Pandemic Disease Threat: Can We Develop A Global Vaccine Policy?	2004	42,595
Alfred P. Sloan Foundation	Communication in Brain Systems	2004	16,000*
Spinal Muscular Atrophy Foundation	Spinal Muscular Atrophy: What is the Molecular Basis of Neuron Loss?	2004	41,403*
The Swartz Foundation	Communication in Brain Systems	2004	17,656*
Verto Institute, LLC	The Biology of Neuroendocrine Tumors	2004	43,244*

*Includes direct and indirect costs.
*New grants awarded in 2004.

DEVELOPMENT

With groundbreaking science as our foundation, Cold Spring Harbor Laboratory has grown from a small institution to a world leader in the biomedical revolution. This progressive change is largely attributed to the increase in financial support received from our many generous benefactors. Without their support, advances in scientific discovery made during the past year could not have been achieved. We are extremely grateful to all who know, value, and support our important work.

Charles 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 program and can sell others.

Charitable Remainder Trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Bequests: Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified.

Conversion of Private Foundation to "Public" Status on Termination: This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a supporting organization of Cold Spring Harbor Laboratory.

Matching Gifts: Many employers will match gifts to Cold Spring Harbor Laboratory and/or the Watson School of Biological Sciences. Please check with your employer to augment your gift.

For additional information, please contact the Chief Development Officer, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6865.

CAPITAL AND PROGRAM CONTRIBUTIONS

January 1, 2003–December 31, 2004

Contributions of $5000 and above, exclusive of Annual Fund

In 2004, Cold Spring Harbor Laboratory received significant support in the form of capital, program, and gifts-in-kind contributions from individuals, foundations, and corporations.

Arrow Electronics, Inc.
Michael Scott Barish Human Cancer Grant sponsored by 1 in 9: The Long Island Breast Cancer Action Coalition
Breast Cancer Help, Inc. and New York State Grand Lodge Order Sons of Italy
The Breast Cancer Research Foundation*
Bristol-Myers Squibb Company
Helen M. Brooks
The New York Community Trust—Cashin Family Fund
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Mr. and Mrs. Robert D. Lindsay and Family*
Estate of Elisabeth S. Livingston
Long Island 2 Day Walk to Fight Breast Cancer
Long Islanders Against Breast Cancer (L.I.A.B.C.)
Thomas P. Maniatis, Ph.D.*
The G. Harold and Leila Y. Mathers Charitable Foundation
Mrs. William L. Matheson
The Maxfield Foundation
Breast Cancer Awareness Day in memory of Elizabeth McFarland
Dr. and Mrs. Walter C. Meier
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The Miracle Foundation
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The Swartz Foundation
Dr. Waclaw Szybalski
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Edwin S. Webster Foundation
Wendt Family Charitable Foundation of Community Foundation Sonoma County*
WALK for Women Breast Cancer Fund
Mark and Karen Zoller
Roy J. Zuckerberg Family Foundation*

*New pledges awarded in 2004.

BENEFIT FOR THE BRAIN
October 30, 2004

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Monsignor Thomas J. Hartman

Committee Co-chairs
Kathy DiMaio and Edward Travaglianti

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William S. Robertson and Banbury Fund

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WOMEN'S PARTNERSHIP FOR SCIENCE
June 27, 2004

Committee Co-chairs
Kristina Perkin Davison
Cristina Mariani-May
Nancy Tilghman

Underwriter
Robert de Rothschild

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Deborah Stevenson

Benefit for the Brain Underwriter Bill Robertson and Honoree Monsignor Thomas J. Hartman

Women's Partnership for Science Co-chairs Nancy Tilghman, Kristina Perkin-Davison, and Cristina Mariani-May

Total	**$27,808,235.64**

ANNUAL CONTRIBUTIONS

Corporate Sponsor Program

The Corporate Sponsor Program continues to provide critical funding for the vigorous Meetings program held at Cold Spring Harbor Laboratory, either at Grace Auditorium on the main 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 eight 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 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, which 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 both the Meetings and Banbury Center pages. Members for 2004 included:

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Association President's Report

In 2004, as the scientific world celebrated 100 Years of Genetics at Cold Spring Harbor, the Cold Spring Harbor Laboratory Association (CSHLA) had a most successful year. Measured in friend-raising and fund-raising terms, our membership grew to almost 1000 strong, and the annual fund, thanks to the loyal support of our donors, surpassed the goal of $1,000,000.00 and reached $1,079,948.52.

The annual fund, as an unrestricted fund, is a critical resource for our young scientists for their continued cutting-edge research. This is consistent with the mission of the Association. The annual fund and other private donations account for nearly half of the Lab's annual funding. It should be noted that Charity Navigator has once again recognized CSHL with their highest four-star rating, for being a fiscally responsible institution. As donors, it is gratifying and reassuring to know that our charitable dollars are going to solid research.

The Association directorship continues to be a hardworking and dedicated group. We welcomed several new and energetic directors to our ranks in 2004: Pien Bosch, Maria Brisbane, Barbara Candee, Kate Friedman, Frank Gundersen, and Raymond Schuville. We bade farewell to Alan Kisner, Mary Alice Kolodner, and Larry Remmel. Each one of them has made significant and lasting contributions to the Lab's outreach and fund-raising efforts. On behalf of all at the Lab, we thank you.

Each year, the Association directors have a series of business and informational meetings. One of the benefits is the lecture series presented by some of the best and most promising scientists and administrators at the Lab. In 2004, we were enlightened by

- Cathy Cormier, Watson School student, who spoke of her current research in Yuri Lazebnik's lab to understand the role of caspases in the molecular mechanisms of apoptosis, a process that is critical for normal cell development and that can cause disease, including cancer, when it malfunctions.
- Art Brings, chief facilities officer, who gave us an update on the Upper Campus building project and satellite operations.

2004 CSHLA Directors

- Wolfgang Lukowitz, assistant professor, who discussed his plant genetics research to identify relevant regulatory functions by isolating and analyzing *Arabidopsis* mutants with aberrations in the early division pattern of the developing embryo.
- Lari Russo, CSHL comptroller, who provided us with an overview of revenue support for research, education, meetings, and capital programs from endowment, government, and private funding.
- Bruce Stillman, president, who gave a summary of the Laboratory's current research and plans for future expansion.

Despite the fact that we directors are lay people and do not understand every scientific word, it has always been educational and energizing to hear firsthand of the scientific work going on here and how it will better our lives in the future.

Sunday, February 29, marked the first Association event for 2004, the Annual Meeting, which is open to all members and their guests. The keynote speaker, Ron McKay, chief of the Laboratory of Molecular Biology in the Basic Neuroscience Program of the National Institute of Neurological Disorders and Stroke, delivered the lecture: *The Science and Politics of Stem Cells*. Dr. McKay's work has importance beyond the intrinsic academic interest in the properties of stem cells and strongly influences strategies for cell- and gene-based therapies for central nervous system disease. The work of his group is widely recognized. He has served on the editorial boards of major journals such as *Neuron*, *Genes & Development*, *Journal of Neuroscience*, and *European Journal of Neuroscience*. The meeting was followed by a hearty cocktail reception.

In March, several directors planned and sponsored a Neighbor/Scientist Cocktail and Dinner Party—with my husband Tom and I, Lucy and George Cutting, Franny and Duncan Elder, and Mary Alice and Michael Kolodner as hosts—to help neighbors get to know CSHL and the scientists. Scientists who attended included Mitya Chklovskii, Ira Hall, Mike Hemann, Leemor Joshua-Tor, Zach Mainen, Shuly Avraham, Alea Mills, and Partha Mitra. All described their work and its implications for human health to the guests.

The Association's major event, *Jazz at the Lab*, was held Saturday, April 17, 2004. Chairs Joe Donohue and Kate Friedman, with their committee, took this event to a sold-out smash hit, which netted well over $100,000. The event featured Diva, a spirited 15-member, all-female big band jazz orchestra. Diva has performed at Carnegie Hall, the Kennedy Center, The Blue Note, Birdland, The Jazz Standard, and jazz festivals around the world. We salute our very generous supporters:

JAZZ AT THE LAB
April 17, 2004

Charles and Helen Dolan and Fritz Coudert at *Jazz at the Lab.*

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Gretchen Tibbits, Mike Obuchowski, Lucy Cutting, Mark Tully, Liz Watson, Morgan Browne at *Jazz at the Lab.*

The year 2004 also marked the formation of the Sustaining Council (SC), thereby formalizing a loyal group of former trustees and directors. The SC, hosted by CSHLA, gathered for the first time on April 20 in Grace Auditorium for a meeting presided over by Dr. Bruce Stillman. Dr. Stillman updated members on the latest science and where research is headed, the success of the Watson School of Biological Sciences, the financial state of the Laboratory, and future growth of the CSHL campus. It is the plan to hold at least one event annually with the group to exchange ideas on how the Lab can best serve the community.

The third annual visit from Goose Hill School first graders occurred May 3–4. Booth presentations included mammalian brains for discussion about human reactions and learning, a variety of plants for learning about how mutations occur, microscopes to examine fruit fly brains, computer monitors featuring protein structures that children viewed through 3D glasses, and mice with different colored coats for learning how scientists model traits.

The 27th annual Dorcas Cummings Memorial Lecture was held Sunday, June 6 as the finale to the 69th Symposium: Epigenetics. The featured lecturer was Dr. David Haig, Professor of Organismic and Evolutionary Biology, Harvard University, who spoke about "The Divided Self: Brains, Brawn, and the Superego." The biggest breakthrough in genetics in the past two decades has been the discovery of genomic imprinting, which allows us to trace genes to the parent of origin. Dr. Haig has been at the forefront of theorizing these developments, arguing that paternally and maternally active genes comprise less than 1% of our total gene count and are far from being cooperative. In fact, they have been shown to be in competition with one another. If Haig's theory holds true, imprinted genes exemplify an extraordinary within-individual conflict, shaking up our fundamental ideas of what it means to be an individual. Following the lecture and reception, many of the visiting scientists were entertained at dinner parties hosted by friends of the Lab in the surrounding community. The event was chaired and organized by Pien Bosch and Lynn Tone. This tradition of community involvement goes back to the early 1920s and is still a favorite event for scientists and local friends alike. Our hosts included:

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George and Lucy Cutting hosted the Partnership Picnic on Sunday, September 12 on the Lab grounds. George and Lucy provided grilled Wild Alaska Salmon, caught and prepared by the Cutting family! A larger crowd than usual with a number of new neighbors and scientists in attendance, a total of 81 guests, made for a perfect autumn day. We welcome all who are interested in joining the Partners for Science program. The intent is for Lab scientists to have an opportunity to meet friends in the community and share some informal socializing, as well as science. For more information, call the Development Office at 516-367-8471.

Director Ray Schuville, as a member of the New York City Committee, hosted a dinner in New York City for private banking clients of JPMorgan Chase that featured Jim Watson and CSHL neuroscientist Tim Tully as guest lecturers. They discussed the potential of current research for eventually understanding autism, schizophrenia, and Alzheimer's disease. The evening was well attended and the guests were quite riveted by the presentation. We thank Ray Schuville for his most generous gesture.

One of the missions of the Association is to help raise scholarship funds for The Mary D. Lindsay Child Care Center. The Center cares for infants through preschool children of Lab employees. Having the Center on the Lab campus is a boost for these families. Each holiday season, the Association hosts a celebration at the Center. Once again, our resident Santa was none other than Association Director John Stevenson. The excitement on the children's faces was something to behold.

George Cutting fishing for wild Alaskan salmon for the Neighbor/Scientist Partner's Picnic in September.

As I retire from the Association, I can't help but reflect on my eight years as a volunteer here (*note: I first became a volunteer when I was 12 years old, thanks to my mother pushing me out the door to "do for others"*). I have seen the Lab grow in national and international prominence, celebrated the 50th Anniversary of the Double Helix, seen the Association substantially increase in membership and funding through the volunteer efforts of the Directors, and witnessed the opening of the Child Care Center and the Cancer Genome Research Center in Woodbury. I could go on for pages. Suffice it to say that it has been a privilege and high honor for me to have a turn at being a volunteer member of the Lab family. Special thanks go to the Development staff, Diane Fagiola, Jean DeForest, and Jan Eisenman. I send best wishes to all for continued success.

Trudy H. Calabrese
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Trudy Calabrese and Joe Donohue

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The President's Council was formed 11 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. The 2004 meeting, co-chaired by Martha Gerry and Charles Harris, took place on May 21 and 22 and explored the topic *The Performance of Thoroughbred Horses: What Makes Them Run*. Our presenting scientists were engaging speakers and leaders in their field: Edward L. Bowen; Douglas Antczak, V.M.D., Ph.D.; Lawrence R. Bramlage, D.V.M., M.S.; Peter J. Timoney, D.V.M., M.S.; and Thomas Tobin, Ph.D., MRCVS, DABT. The following were members of the 2004 President's Council:

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In 2004, we were pleased to receive notification of gifts from the estates of Georgette Spingarn and Adele Diaz. We also received additional funds from the estates of Elizabeth Livingston and Florence Strelkowski. In addition, we established a new charitable gift annuity program whereby we can offer donors an excellent return for the rest of their lives on their contributions. At the same time, the donor is qualified for an immediate federal income tax charitable gift deduction and lower capital gains taxes on gifts of securities bought at prices well below current levels.

In the spring and fall of 2004, Estate and Tax Planning seminars were held at the Cancer Genome Research Center in Woodbury. Those who attended were shown many ways whereby creative and customized planned giving strategies offer worthwhile tax and income benefits while at the same time providing the funds necessary to make a difference in the health and well-being of their children, grandchildren, and future generations.

Undergraduate Research Program

Founded in 1959, the Undergraduate Research Program allows college students from around the world to participate in hands-on, inquiry-based avenues of exploration. This educational approach increases students' understanding of science, nurtures their enthusiasm about science, exposes them to the excitement of scientific discovery, and stimulates their curiosities, while interesting them in pursuing research careers or other science careers. Twenty-two participants composed of 11 men and 11 women stemming from Canada, Israel, Korea, Poland, the United Kingdom, and the United States spent 10 weeks during the summer at Cold Spring Harbor Laboratory conducting research under the guidance of staff scientists.

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