ANNUAL REPORT 2008

COLD SPRING HARBOR LABORATORY

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Front cover: Visualizing gene expression in a living cell. Image of a living human cell shows a cluster of active genes in the nucleus (*bright green region*), and the protein product that is encoded by these genes, in the cytoplasm of the cell (*blue peroxisomes*). Photo provided by the Spector Laboratory.

Section title page photos: Constance Brukin

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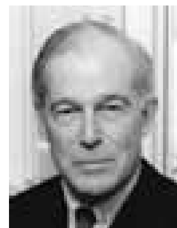

Henry Wendt III
Friday Harbor, Washington

*Through November 2008
**Deceased (2009)

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PRESIDENT'S REPORT

On the same day in February 1809, two individuals were born, who, by virtue of their intellect and the course of events, would greatly influence contemporary thinking and establish principles that would be debated long after they were gone. Abraham Lincoln, through skillful political leadership and with sound moral compass, saved the union of the United States and set in motion what would be a longer than expected struggle for equality in America. Charles Darwin expanded on ideas about evolution that emerged in the early part of the 19th century to articulate a revolutionary approach to thinking about how species arise and evolve. In 2009, Cold Spring Harbor Laboratory will take special pride in celebrating the achievements of Charles Darwin, recognizing that our work is connected to his in a direct line of descent, for the very beginnings of the Laboratory can be traced to the scientific urge "to investigate experimentally the origin of species."

Those words were used in a proposal for the construction of a permanent Station for Experimental Evolution at our current location in the early years of the 20th century. The goal was to apply Darwin's theory and test Mendel's laws in animal and plant breeding experiments. When such a Station opened its doors in 1904, thanks to a grant from the Carnegie Institution of Washington, the idea that gave rise to Cold Spring Harbor Laboratory was already 14 years old. At an old fish hatchery on the edge of an intertidal zone, a field station of the Brooklyn Institute of Arts and Sciences had been established in 1890 to study how life, per Darwin's theory, had adapted to fill a diversity of ecological niches.

The Laboratory has itself evolved since its days of affiliation with the Brooklyn Institute and the Carnegie Institution. Although evolution in the natural world is selected from a wide variety of nondirected mutations, in the case of our institution, change has been deeply purposeful. From its beginnings in the observation-based study of evolution, to its focus on genetics under Charles Davenport beginning in 1904, to its historic role in the 1940s and 1950s in incubating molecular genetics and molecular biology under the leadership of the geneticist Milislav Demerec, Cold Spring Harbor Laboratory has taken the lead in setting the agenda for the advancement of biological science.

Progress in science depends on a variety of factors: intellectual and conceptual breakthroughs, technological leaps, firm financial backing, and lots of hard work by scientists who have a passion for what they do. Advances cannot be predicted, and sometimes serendipity is a real factor in our movement forward. But the experience of more than a century demonstrates that the process of discovery is continuous and that it exerts an inexorable force on institutions such as ours to adapt or risk falling away from the leading edge in generating knowledge.

The current phase in our evolution is being given palpable, physical expression in the six new interconnected laboratory buildings that comprise our Hillside Research Complex, now nearly finished. These beautifully conceived and executed structures underlie the future evolution of research at the Laboratory. They announce, first, our intention to build on our expertise in cancer research, an area in which we have earned great respect since 1968, thanks to the vision of James D. Watson. Among the new structures is one that will be home to the CSHL Cancer Center and house important research that investigates the relationship between cancer genetics and therapeutic strategies. An adjacent building will focus on tumor microenvironment and metastasis, both areas that are key to developing new therapeutic strategies.

There are two other messages that these new buildings send regarding CSHL's evolution. One is about how we are adapting to changes in the way science is performed; the other is about how biological science advances along an endless frontier, to use a phrase made famous by Vannevar Bush, the father of modern American public science policy.

No one involved in biological research needs to be reminded of the impact that advances in information technology have had on the pace and nature of the work. Science moves ahead much more rapidly today than it ever has, and in our fields of biology, much of the research is now performed by groups larger than ever before, comprised of members representing a diverse range of fields and disciplines. Multidisciplinary team science has come to dominate the research landscape and CSHL has adapted well to its advent. Our Meetings and Courses and Banbury programs facilitate such an approach to team-based science because CSHL continues to be the crossroads for life scientists across many disciplines, a place of intellectual ferment and vigor at the very center of the action.

The author lists on many of the hundreds of research papers published by our faculty this past year offer evidence of the multidisciplinary richness of the science that we do. CSHL is a place where collegiality thrives, a

fact that many Laboratory visitors comment upon each year. One can see it at the in-house lectures given every week on our campus, and perhaps most vividly at the Friday noontime lectures, which more often than not attract overflow audiences of faculty and postdocs listening intently to results obtained by their neighbors, on topics entirely outside of their own fields of expertise. Regular exposure to the ideas of others is a condition of successful team science at CSHL, and I believe it is at the core of great discoveries.

Carefully planned strategic investments in our research program also aid in realizing potential synergies and in taking advantage of new opportunities, many of them interdisciplinary. Perhaps the most important of our investments this past year involved two new areas of research, in neuroscience and quantitative biology. The neuroscience program is a case study of how disciplines once regarded as distinct from one another yield insights that constitute new sources of institutional strength. For instance, a wholly independent line of work centering on the human genetics of cognitive dysfunction has emerged in our neuroscience effort and is now merging with separate studies on brain anatomy, neuronal development, and behavioral analysis of normal cognition.

Our genetics research, which is already shedding new light on the complex genetic causation of illnesses such as autism, schizophrenia, and bipolar depression, emerged from techniques developed at CSHL to study the genetics of cancer and adds a new dimension to the neuroscience effort at CSHL to understand long-term memory and cognitive processes such as attention, decision making, and working memory. That effort, which will expand into new buildings at the Hillside Research Complex, seeks to understand the structure and function of neural circuits whose activity makes possible complex behaviors and the cellular biology underlying these circuits. It also explores how the brain as an ensemble of circuits responds to external stimuli and how the processing of these stimuli serves as the basis of cognition. What is now particularly pleasing is that the genetics of psychiatric diseases in humans is starting to merge with basic studies on rodent cognition and neuronal development, a program that has been built up over the past five or so years. The genes implicated in autism, schizophrenia, and depression are becoming focal points for understanding what they do in the normal brain and how alterations in these genes affect complex cognitive behaviors.

The latter thrust, in systems neuroscience, is one area in which mathematical and statistical insights are certain to help drive research forward. We are reminded of “the endless frontier”—the startlingly new approaches to science that the human mind continues to generate that exert a kind of positive adaptive pressure. Such adaptation is no more apparent than in the requirement in all our research programs for sophisticated, quantitative analysis of large data sets.

Events in 2008 realized a major step forward with the hiring of the first members of what will be a distinctive feature of the research program at the Laboratory—our new Center for Quantitative Biology. A brainchild of Michael Wigler, it was made possible by forward-looking and sizable grants from the Simons and Starr foundations. The Center will become the home base for outstanding scientists who are primarily focused on mathematical and computational approaches to biology, applying their insights in the formulation of research hypotheses pertinent to all of the areas in which the Laboratory specializes: cancer, neuroscience, and plant biology. One particularly interesting aspect of this area of research that has already emerged from the studies of the center’s first new faculty member, Gurinder (“Mickey”) Atwal, is the quantitative analysis of mutations in the human population, informing us in new ways about the processes of evolution and natural selection.

The Laboratory, then, is succeeding in its missions as it continues to adapt, both proactively and in response to external events. One of the major external events that has already altered our behavior during the last year has been the global crisis in the financial system that clearly will impact our work over the near term. Our significant reliance on fund-raising to support the cutting-edge research of our very productive faculty, including many in the earliest stages of their independent careers, will be a constant challenge.

Ironically, this crisis is occurring at a moment when a new president has been elected, fulfilling the dreams of Abraham Lincoln and all too many who have followed. It is a time of major crisis that rivals some of the most serious in the nation’s history. Fortunately, the new administration has renewed this nation’s commitment to including science in its policy making, while recognizing that science is not the only arbitrator of a vibrant political system.

On a more practical note, the public sector’s commitment to funding scientific research will in part reverse the significant cuts in funding that we have endured during the past seven years. Because it is not possible to predict the course of macroeconomic events any more than discoveries in science, we have already taken strong measures at the Laboratory to trim, save, and conserve wherever possible. But confidence in the future is not out of place with a new federal administration in place, our supporters continuing to recognize the importance of our science, and our scientists continuing to be among the most productive and innovative in their fields of endeavor.

HIGHLIGHTS OF THE YEAR

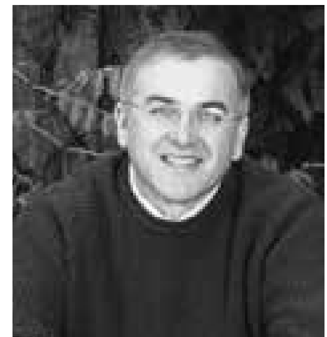
Research

Research at Cold Spring Harbor Laboratory continues to flourish. This will surprise no one in the community of scientists, within which our reputation ranks among those of the very best life science research institutions worldwide. This fact was reflected once again in an annual survey by Thompson Scientific's *Essential Science Indicators*, which reported in 2008 that CSHL is in the top 1% of institutions most cited in published research and that our faculty is ranked by peers among the top three in terms of its influence in shaping the fields of molecular biology and genetics. Much of this is due to the sound guidance of our research administration, which consists of a very effective team headed by Director of Research David L. Spector and includes Research Executive Committee members Greg Hannon, Scott Lowe, and Tony Zador, who receive outstanding administrative assistance from Sydney Gary and Walter Goldschmidts.

The Laboratory's achievements in 2008 are particularly noteworthy when considered in the context of the current funding environment. Against a backdrop of limited resources and a contraction in federal grant funding, scientists in all of the areas in which CSHL concentrates—cancer, neuroscience, and plant biology—reported a steady stream of significant research results throughout the year. Although these are comprehensively described on our website, it is useful to briefly review a few among the many to illustrate the richness of our scientists' cumulative achievements in 2008 and the continuing overall success of our research program.

Compensating for a Missing Gene in SMA

The neuromuscular disease called spinal muscular atrophy (SMA) can lead to death in children before the age of two. It is traced to a protein deficiency caused by mutations in the gene *SMN1*, which in turn leads to serious damage in growing nerve cells and the muscles that these cells control. This past year, Adrian Krainer and colleagues at CSHL and Isis Pharmaceuticals induced cells to replenish the deficient protein by activating a poorly expressed relative of *SMN1* called *SMN2* that resides in the human genome. This was done by inducing alternative splicing of a second copy of the *SMN2* gene so that it now included a missing piece that makes it more active and able to prevent disease progression. Adrian and his team sought to change the splicing by introducing synthetic molecules called antisense oligonucleotides (ASOs). The team injected their most potent ASOs into mice that had an added, human version of *SMN2*. As they had hoped, the gene produced much more of the RNA for the critical protein, including the section that is usually omitted in *SMN2*, in tissues where the ASOs accumulate. They now seek to determine whether the ASOs really benefit growing animals with SMA and how and when they should be administered to affect the nervous system. This research follows a decade or more of fundamental research by Adrian and his colleagues regarding the understanding of control mechanisms of RNA splicing. Without this knowledge, the advances in therapeutic strategies would not be possible.



A. Krainer

Causal Link between a Tumor-suppressor Gene and Liver Cancer

S. Lowe

In a project that attests to the spirit of cooperation among our laboratories, no fewer than five CSHL teams joined forces in 2008 to confirm that a gene called *DLC1* is a tumor suppressor. In an effort spearheaded by Scott Lowe, they demonstrated in living mice that deletion, loss, or inactivation of the gene precipitates events culminating in an aggressive type of liver cancer closely related to common human epithelial cancers of the liver. Tumor suppressor genes have a vital role in intracellular signaling networks that protect against uncontrolled cell growth and proliferation. Such genes can be rendered inactive by a variety of DNA alterations. *DLC1*, a gene whose acronym reflects prior suspicions that it was "deleted in liver cancer," was known to be located in a region of chromosome 8 that has been observed to be missing in past studies of mammalian liver cancer cells. Importantly, the team's success in tracing the pathway by which *DLC1* functions in both healthy and pathological states suggests a highly specific new target for future anticancer drugs. Using a mechanism called RNA interference (RNAi) to control the expression of specific genes, the team effectively turned off the DLC1 "switch" in living mice cells and, in so doing, was able to isolate one particular signaling intermediary whose presence was both necessary and sufficient to set the cell on an uncontrolled growth path. This essential molecular intermediary, a potential drug target, is called RhoA.

Role of Rare Gene Mutations in Schizophrenia

J. Sebat

In 2008, Jonathan Sebat and colleagues from the University of Washington and the National Institute of Mental Health contributed an important finding to our rapidly growing knowledge about the genetics of schizophrenia. They identified multiple, individually rare gene mutations in individuals with this devastating illness that may help to explain its causation in at least a subset of cases. Jonathan and the team screened the genomes of patients and healthy controls for gene copy-number variations (CNVs)—a type of structural variation in the genome characterized by the presence of either too many or too few copies of particular genes. They found that deletions, disruptions, and duplications of normal genes were three to four times more frequent in patients compared with controls. The study suggested that rare mutations not only are common in schizophrenia, but are also often quite powerful. In a preliminary effort to discover the role of these rare mutations in disease etiology, the team found, intriguingly, that more than half of the disrupted genes were involved in pathways involved in neuronal development and regulation.

Rethinking the Neural Code

A. Zador

Anthony Zador, a neuroscientist who is interested in how neural circuitry in our brains gives rise to astonishing system properties such as consciousness and perception, performed a simple yet powerful experiment this past year that produced a surprising and important result. Tony wanted to determine the shortest possible time interval between neuronal signals or "spike trains" that an animal's cortex could distinguish and use to make a decision. The remarkable answer, observed in the auditory cortex of living rats, proved to be

three one-thousandths of a second (3 ms). These data provide support for an alternative theory of how information is processed in the brain, sometimes called the “neural code.” The prevailing theory is based on the observation that neurons spike more quickly when they are transmitting information. This supports a “rate code” model, which stipulates that information is contained within the spiking rate of the neuron. However, Tony's experiment lends credence to a “timing code” model, wherein information is encoded within the precise pattern of spiking, which can be deduced by examining how spikes are distributed over time.

How the Brain Decides What to Believe

A. Kepecs

The laboratory of Adam Kepecs is also making important and original contributions to the study of the brain’s system properties. Adam and his colleagues point out that even the simplest decisions involve the integration of sensory and memory information with emotional and motivational attributes, requiring the concerted action of millions of neurons across brain regions. Their current work seeks to elucidate the neurocomputational principles of decision making and attempts to capture elusive attributes such as emotion, motivation, or confidence. This past year, Adam, with Zachary Mainen and colleagues at CSHL, discovered neural signals for confidence in decision making in the rat prefrontal cortex. Their study suggests that confidence estimation is a fundamental information-processing mechanism in the brain, not a complex function specific to humans but a core component of decision making. They speculate that it is found throughout the animal kingdom, shared widely across species, and not strictly confined to those, such as humans, that are self-aware.

Epigenetic “Reprogramming” of Plant Cells

Transposons are bits of DNA that can jump around in the genome and disrupt normal gene function and regulation. Under normal conditions, genomic chaos, found to occur in cancer and other diseases, is prevented in various ways, among which are a series of mechanisms that scientists call epigenetic. These mechanisms modify the expression of genes without altering their DNA sequence. Among the pioneers in the study of epigenetics is Robert Martienssen, a worthy successor to the late Barbara McClintock, the Nobel Laureate who, in discovering “controlling elements,” vividly demonstrated at CSHL what we could learn about genetics by studying plant systems. This past year, Rob’s team continued their ambitious project to map the changing epigenetic landscape of “immortalized” or continuously dividing plant cells. They succeeded in describing epigenetic alterations that allow transposons to escape normal regulatory controls. Transposons in plant genomes are normally rendered inactive via RNAi, which is orchestrated by small interfering RNAs (siRNAs) that target heterochromatin (densely packed, genetically inactive regions of DNA). In immortalized plant cells, Rob’s team found that epigenetic changes resulting in a loss of heterochromatin and transposon “reactivation” are not due to a loss of proteins regulating heterochromatin. They found instead that they were due to a change in the population of siRNAs produced in the continuously dividing cells. This suggests that siRNA-driven heterochromatin restructuring may lead to the formation of “epialleles”—epigenetic variations in gene expression patterns that stem from the creation of particular states in the chromosomes of cells, thereby changing the patterns of gene expression in related cells.

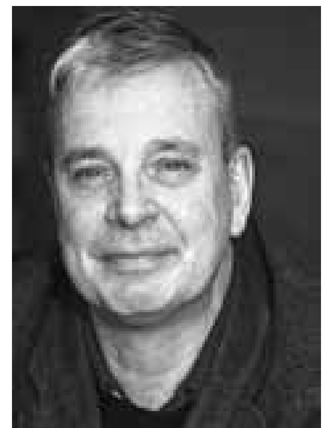
R. Martienssen

Small RNAs Involved in Transmission of Epigenetic Information: Genome Defense

G. Hannon

One way in which epigenetic information is known to be passed from parent to offspring is through the pattern of chemical "caps" added onto certain "letters" of the DNA sequence, ensuring that the sequence is "silenced." In some cases, enzymes that add these caps are guided to DNA by small RNA molecules. Gregory Hannon, a leader in the study of small RNAs, discovered (in part, with Leemor Joshua-Tor at CSHL) the cellular machinery that "dices" and "slices" double-stranded RNAs into gene-regulating single-stranded mi- and si-RNAs. Greg's team has now discovered that a class of small RNAs carries epigenetic information and has demonstrated how, in one instance, they pass on the trait of fertility from mother to offspring in fruit flies. This was only one of several discoveries made by Greg's team this year, most involving small RNAs, which, he has observed, are far more diverse as a class than initially suspected and act in more ways than anyone imagined when they were first described about a decade ago. In 2008, the Hannon team described a new class of small RNAs that partner with the protein Argonaute 2. Using advanced sequencing technology, they found that these small RNA partners modify gene activity and suppress transposable elements, thus serving as a genome defense mechanism in cells that are destined to become the germ line for the next generation. These studies have helped to explain old and intriguing observations that when certain strains of animals are mated, their offspring are not fertile, a process called hybrid dysgenesis.

New Perspectives on "Noncoding" Portions of the Genome


T. Gingeras

Discovering where and how functional information is stored in genomes represents a frontier of research that underlies all life sciences and clinical research. Without this information, the biological and clinical effects of disease-causing mutations in humans and other organisms can only be partially understood. Recently, detailed analyses regarding which genomic segments are transcribed into RNA and the functional roles of these RNAs have revealed the fact that our current models of how genomes are organized and regulated remain rudimentary. As one of the leaders of the National Institutes of Health's project ENCODE (*ENC*yclopedia *O*f *D*NA *E*lements), Thomas Gingeras—who rejoined our faculty in 2008—has determined that almost all of the human genome can be transcribed into RNA and that most of these RNA products are not made to be translated into proteins. Rather, non-protein-coding RNAs are used in a variety of functions ranging from regulation of expression of protein-coding genes to acting as scaffolds upon which large protein complexes are assembled. As reported in a front-page feature in the *Science Times* section of *The New York Times* on November 11, Tom's research is helping scientists to significantly revise long-standing notions about such basic questions as what constitutes a gene.

Histone Modifications with "Personality"

Michael Zhang's group has developed a series of computational tools that make use of statistical pattern-recognition techniques to identify exons, promoters, and posttranslational

modification signals in large genomic DNA sequences. They also study alternative splicing of exons and collaborate with other labs to characterize splicing enhancers and silencers. This past year, Michael's group was part of a team that published a comprehensive analysis of modification patterns in histones. Using a new technology called ChIP-Seq, they identified 39 histone modifications, including a "core set" of 17 modifications that tended to occur together and were associated with genes observed to be active. The various modifications showed distinctive "personalities," each preferentially associating with particular regulatory regions of genes. They developed a better promoter prediction algorithm by taking into account such epigenetic information. They also worked with Professors Li at the University of Chicago and Krainer at CSHL to discover that one of the steps in turning genetic information into proteins leaves genetic fingerprints, even on regions of the DNA that are not involved in coding for the final protein. They estimated that such fingerprints affect at least a third of the genome and suggested that although most DNA does not code for proteins, much of it is nonetheless biologically important enough to have persisted during evolution. In addition, with Adrian Krainer, Michael's team succeeded in defining the regulatory networks of two related tissue-specific (brain and muscle) splicing factors, Fox-1 and Fox-2.

M. Zhang

Three CSHL Studies Appear in Cell*—in a Single Issue*

Three separate research teams each led by our faculty reported results in the November 26 issue of the journal *Cell*—a reflection of the caliber and relevance of the work that is routinely performed at the Laboratory. It is unusual for multiple, unrelated studies emanating from the same institution to appear in a single issue of this prestigious journal, and it is truly remarkable for three teams to report on different projects in the pages of a single issue, given the relatively small size of our faculty, now at 47. A team consisting of Scott Powers, Gregory Hannon, W. Richard McCombie, Michael Wigler, and corresponding author Scott Lowe reported 13 new tumor-suppressor genes in liver cancer, which they revealed using a powerful new approach to validate linkages between suspect genes and their functional contributions to cancer. A second CSHL study in the same issue of *Cell* by Senthil Muthuswamy and colleagues demonstrated that normal function of the protein Scribble allows breast epithelial cells to form duct-like structures and resist cancer formation. They were also able to show that when Scribble stops functioning, the tissue loses its shape and the earliest stages of cancers ensue. The results constitute first steps toward identifying a new class of molecules and pathways that can be targeted by anticancer therapies to prevent precancerous lesions from turning into malignant tumors. In the third CSHL paper, a team in David Spector's lab shed light on possible functions of noncoding RNA molecules, announcing the discovery of a previously unknown mechanism in the nucleus that sends different parts of the noncoding RNA molecule MALAT1 to different cellular destinations.

Cold Spring Harbor Laboratory Board of Trustees

The Board of Trustees welcomed five new members: Leo A. Guthart, founder and manager of Topspin Partners, a venture and private equity firm based on Long Island; Thomas Lehrman, president and founder of Boliven, LLC, an internet start-up focusing on innovation, consulting, and venture investing in technology businesses; Marilyn Simons, Ph.D., president of the Simons Foundation, which funds basic research and education programs in mathe-

matics and physical and life sciences; and Paul Taubman, managing director of Morgan Stanley. Charles L. Sawyers, M.D., Howard Hughes Medical Investigator and head of the Human Oncology and Pathogenesis Program at Memorial Sloan-Kettering Cancer Center, was elected a Scientific Trustee. In addition, the ranks of our honorary trustees grew with the addition of Norris Darrell, Senior Counsel, Sullivan & Cromwell LLP and CSHL Chancellor Emeritus James D. Watson, Ph.D.

On behalf of CSHL and the Board of Trustees, I thank departing Scientific Trustee Robert E. Wittes, M.D., for his service. Dr. Wittes was elected to the Board of Trustees as a Scientific Trustee in February 2004 and served on the Tenure and Appointments Committee from 2004 to 2008 and the Research Committee from 2004 to 2008. He stepped down in November 2008.

We are grateful to members of the Cold Spring Harbor Laboratory Association (CSHLA), who came together often in 2008, cultivating new friends and raising funds to support the research of scientists who are at the early and most innovative stages of their careers in research at CSHL. Despite the difficult economy, CSHLA members raised more than $1.1 million for the annual fund.

Highlights from the CSHLA this past year included dancing to the latest Latin rhythms, an evening of education about skin stem cells, and a special thank you to major donors. The April *The Lab Goes Latin* dinner and dance event was a lively change from our traditional spring jazz benefit. We applaud co-chairs Lisa and Tim Broadbent and Kate and John Friedman for the tremendous energy and creativity that they put into this successful event. In early June, many of our new association members hosted dinner parties in their homes for visiting and CSHL scientists as part of the annual CSHL Symposium's Dorcas Cummings public lecture. Researchers and guests from the local community enjoyed the presentation on skin stem cells given by Elaine Fuchs, Ph.D., head of the Laboratory of Mammalian Cell Biology and Development at The Rockefeller University.

The rain did not dampen attendance by a record number of Association members and major donors at a lovely reception held in their honor at the home of CSHL Trustee Stephen Lessing and his wife Sandra in nearby Lloyd Harbor, New York.

I thank Pien Bosch, who served with exceptional dedication and success as President of the CSHL Association in 2007 and 2008. I look forward to the continued involvement of Pien and her husband Hans at CSHL and welcome all of the new friends that the Association directors and members introduce to the CSHL community.

Research and Education Management

We are proud of our continuing commitment to strategic and fiscal management of our research and education programs, which must evolve constantly to meet the challenges of contemporary science and society. This year, we established an independent Scientific Advisory Council (SAC) that is comprised of scientific leaders from top research universities and institutes. SAC will advise this institution on important issues related to research and education that will be necessary to maintain CSHL as a world leader. The nine-member SAC is chaired by Frederick W. Alt, a Howard Hughes Medical Investigator, member of the National Academy of Sciences, Professor of Pediatrics at Children's Hospital Boston, and Professor of Genetics and Scientific Director of the Immune Disease Institute at Harvard Medical School. Fred taught the influential molecular cloning course at CSHL in the 1980s. Other SAC members include Cornelia Bargmann, The Rockefeller University; David Botstein,

Princeton University; Joanne Chory, Salk Institute for Biological Studies; Carol Greider, Johns Hopkins School of Medicine; Leonid Kruglyak, Princeton University; Markus Meister, Harvard University; Tony Pawson, Samuel Lunenfeld Research Institute; and Max Wicha, University of Michigan.

CSHL hosted the inaugural conference of the iPlant Collaborative, a National Science Foundation–funded $50 million project to create a virtual center in cyberspace for plant sciences, researchers, and students. The kickoff conference, entitled “Bringing Plant and Computing Scientists Together to Solve Plant Biology’s Grand Challenges,” took place April 7–9, 2008. In addition to CSHL, institutions in the iPlant Collaborative include the University of Arizona, Arizona State University, the University of North Carolina at Wilmington, and Purdue University.

CSHL Education Programs Expand

This year, through the skillful leadership of David Stewart, Executive Director of the Meetings and Courses program, we established a wholly owned subsidiary called Cold Spring Harbor Conferences Asia, to develop and operate an annual program of scientific conferences modeled after the CSHL meeting format. Our own in-house legal counsel John Maroney and his staff have been instrumental in this initiative.

Located on a 25-acre campus beside Dushu Lake in the eastern part of the city of Suzhou, which is in the Golden Triangle region of Shanghai on the lower reaches of the Yangtze River, the 600,000-square-foot ultramodern conference center and hotel currently under construction is funded by the Suzhou Industrial Park.

Two views of the proposed China Conference Center

Cold Spring Harbor Conferences Asia appointed a distinguished Scientific Advisory Board of 20 academic scientists from Asia, Europe, Canada, and the United States, which held its inaugural meeting in Suzhou in October 2008. Cold Spring Harbor Asia plans to begin a program of international scientific conferences at the Suzhou Dushu Lake site in 2010.

Our foray into Asia recognizes the global nature of advanced science and builds on existing strengths of the CSHL Meetings and Courses program, which currently attracts more than 8000 scientists to our campus each year for exceptional scientific exchange and discussion, free of commercial or regional/national bias. We have a successful record of international educational partnerships and collaborations in Europe that include programs with the European Molecular Biology Organization in Germany and the Wellcome Trust in England.

The year 2008 was an inaugural one for the CSHL Personal Genomes meeting, whose time had finally come, thanks to advances in technology that are now making economical and efficient sequencing a reality. Our Chancellor Emeritus James D. Watson was one of only four whose individual genome had been sequenced and assembled at the time of the meeting. He and J. Craig Venter were honored at CSHL's third annual Double Helix Medals Dinner on November 6 for making their personal genome sequences available to the public. As more genomes are sequenced and analyzed, the CSHL Personal Genomes meeting will surely become an increasingly important venue for discussion linking personal genome data of humans and the genomes of many other species, as well as the societal issues associated with the accumulation of such personal data.

In September 2008, together with New York City Department of Education Chancellor Joel I. Klein, we officially opened an important extension of our DNA Learning Center, called the Harlem DNA Lab, at the John S. Roberts Education Complex in East Harlem. The Harlem DNA Lab is CSHL's first New York City–based education facility. It is amazing to think that it was only 20 years ago this year that we opened the Dolan DNA Learning Center facility in the village of Cold Spring Harbor. Since then, CSHL has provided hands-on educational experiences in genetics to more than 325,000 children and teachers on Long Island. During

Chancellor Joel Klein was a featured speaker at the Harlem DNA Lab opening in September.

the last two decades, Dolan DNA Learning Center Executive Director David Micklos and his team have profoundly influenced the way in which biology and genetics are taught in schools, not only in New York, but around this country and around the globe.

Students get hands-on education in genetics at the Harlem DNA Lab.

The Harlem DNA Lab is now bringing the latest knowledge and cutting-edge tools and techniques of modern biology to New York City's middle and high school students and teachers. With generous funding from the Howard Hughes Medical Institute (HHMI) and initial support from the Dana Foundation, Jerome L. Greene Foundation, The Goldman Sachs Foundation, and William Townsend Porter Foundation, New York City's 8th and 9th grade science teachers will receive professional development in genetics and biotechnology in order to boost student performance in science.

In this anniversary year for the DNALC we also cut the ribbon on the Laurie L. Landeau Multimedia Studio to support the next phase in the development of the DNALC's Biomedia Group as a world leader in biology education. The studio contains the latest equipment for high-quality video production and is designed with separate sets for interviews, news reporting, and lab demonstrations. Many thanks to CSHL Trustee, Education Committee chairperson, and long-time friend Laurie J. Landeau, V.M.D., for making this possible.

The year 2008 was a multiple anniversary year for our Chancellor Emeritus James D. Watson, who not only celebrated his 80th birthday and 40-year wedding anniversary with Liz Watson, but also marked 40 years of dedicated contributions to the Laboratory. His imprint on CSHL spans every aspect of the Laboratory's life, ranging from financial stability, to beautiful landscapes, an expanded research program, the growth of professional education programs, and the establishment of the Watson School of Biological Sciences and the Dolan DNA Learning Center.

Liz Watson marked the year with the release of her new book *Grounds for Knowledge*, published by CSHL Press, which, under the leadership of John Inglis, continues in its commitment to publishing excellence. The publication coincided with the designation by the Public Gardens Association of America of the CSHL Bungtown Road campus as a botanical garden. Liz's book, filled with beautiful prose and the stunning photography of CSHL Director of Facilities Peter Stahl, showcases the landscapes of our shoreline location. As a campus community, we celebrated and joined with friends in the international scientific community to reminisce with Jim and Liz on several occasions this year, including an employee picnic honoring the Watsons in August 2008. Groundbreaking on the Hillside Campus expansion project began in 2005, but this year, we witnessed the most dramatic progress yet in this monumental capital project that will increase our research capacity in cancer, neuroscience, and bioinformatics by nearly 40%. At the beginning of the year, only the foundations for the six-building research complex were in place. By year's end, all of the internal piping and wiring were installed; the concrete superstructure was complete, exterior block walls were erected, and the roof structure and sheathing were in place, enclosing the buildings and ensuring that interior work continued through the winter months.

All the work on building structures could readily be seen at a glance as it progressed. Much less obvious was the enormous amount of work taking place inside the structures. Electricians and steamfitters worked hard, installing electrical and mechanical systems, including the two high-pressure boilers that will ultimately heat the six-building complex. These two areas were by far the most complex components of the project, and the impressive progress made in these areas is essential to the timely completion of the Hillside Campus.

The final touches were also put on the new chiller plant, and much of the underlying infrastructure—drainage systems, electrical conduit and wiring, water and sewer piping—was installed underground.

The Nassau County Chapter of the New York State Society of Professional Engineers presented CSHL with the Project of the Year Award for the engineering and innovative environmental design of the storm water management system for the Hillside Campus. CSHL was recognized for achieving a balance between development and the environment. The unique and functional storm water management system not only is effective at protecting the surrounding ecosystems, but also provides a visually pleasing backdrop for the Laboratory.

We thank Vice President and Chief Facilities Officer Art Brings and the Facilities Department for all of these great results and the minimal disruption to our daily operations and quality of life on campus as the work has proceeded.

Awards and Honors

L. Joshua-Tor

CSHL professor and Dean of the Watson School of Biological Sciences Leemor Joshua-Tor was one of 56 newly named HHMI Investigators in 2008. She joins approximately 300 HHMI Investigators in the Institute's flagship program, who lead HHMI laboratories at 64 institutions. In addition to Leemor, HHMI Investigators at CSHL include Gregory J. Hannon, Ph.D. and Scott Lowe, Ph.D.

For leadership in computational approaches and leveraging emerging sequence technology to link candidate genes and their function with agricultural traits and germplasm improvement, CSHL adjunct assistant professor Doreen Ware received the 2008 Scientist of the Year Award for the North Atlantic Area from the United States Department of Agriculture.

D. Ware

CSHL postdoctoral fellow Alexei Aravin was a 2008 finalist in the second annual New York Academy of Sciences Blavatnik Awards for Young Scientists. Sixteen young scientists from the New York tristate area were selected for their outstanding work as postdoctoral fellows and young faculty members. Five winners were chosen after three rounds of review of the 16 finalists, who represented a wide scientific and institutional spectrum in engineering, physics, and biology at nine different institutions in New York, New Jersey, and Connecticut.

Thompson Scientific's *Essential Science Indicators* ranked the research conducted at CSHL among the most cited in the world. The analysis, reported in the January/February issues of *Science Watch*, placed CSHL in the top 1% of institutions most cited and identified our research as having significant impact on molecular biology and genetics research from 2002 to 2006. This follows the previous ranking of CSHL by *Science Watch* as the top institution in the world in molecular biology and genetics during the decade from 1993 to 2002, based on citation impact.

I was honored to be elected this year to the American Academy of Arts and Sciences, joining other inductees who included CSHL friend and science philanthropist James H. Simons.

At the 2008 Watson School of Biological Sciences Commencement Convocation on April 13, we conferred the degree of Doctor of Philosophy to Hiroki Asari, Rebecca Bish, François Bolduc, Monica Dus, Angélique Girard, Christopher Harvey, and Wei Wei. Claire Biot and Adrienne Jones received the Master of Science degree. The Watson School also bestowed honorary degrees on three distinguished recipients. The first is one of the world's most successful entrepreneurs, who established the Allen Brain Institute that has mapped the gene architecture of the brain—Microsoft

Watson School Commencement Convocation

cofounder Paul G. Allen. The remaining two include the Nobel Prize–winning neuroscientist Dr. Eric Kandel and a clinician whose ability to translate the complexities of the brain's biology into household concepts has made him a cultural icon: Dr. Oliver Sacks.

The 2008 incoming class is the 10th of the Watson School and represents a record number of students—a total of 15—who come to Long Island from Australia, France, Germany, Italy, South Korea, Russia, and Turkey, in addition to the United States. This year, we also established the Gonzalo Rio Arronte Fellowship, to be held by a qualified Mexican graduate student. Candidates for the fellowship will be preselected by the Universidad Nacional Autónoma de México and the Fundación Gonzalo Río Arronte and presented to the Watson School Admissions Committee, which will make the final decision on admission. The fellow will be expected to have a role in the development of genomic sciences in Mexico upon completion of his or her graduate and postdoctoral training.

For the seventh consecutive year, CSHL received the highest possible rating from Charity Navigator for its financial organizational efficiency and capacity.

As a private nonprofit research and education institution, we are proud to be recognized for achieving the highest standards of financial responsibility. These standards distinguish CSHL as an institution prepared to meet the expectations of public and private donors, who demand accountability, transparency, and quantifiable results from their contributions to nonprofit organizations. Thank you to CSHL Chief Operating Officer Dill Ayres and Comptroller Lari Russo for their commitment to ensuring the highest standards for CSHL.

Development

With the hard work of the Development department, led by Charlie Prizzi, Cold Spring Harbor Laboratory's fund-raising efforts saw much success in 2008. The $200 million goal for the Hillside Campus Campaign was surpassed and more than $41 million was raised in unrestricted and program support. Generous philanthropic gifts resulted in the establishment of the Center for Quantitative Biology at Cold Spring Harbor Laboratory, which will be based in the newly constructed David H. Koch Laboratory.

The Simons Foundation and The Stanley Medical Research Institute have continued to fund programs aimed at uncovering the genetics behind autism, schizophrenia, and bipolar disorder. Additionally, a generous commitment was made by HHMI to help expand the laboratory space for the CSHL Meetings and Courses program so that more scientists will have the opportunity to participate in our world-renowned educational programs.

On behalf of CSHL, our Board of Trustees, and our Development department, I would like to acknowledge all those who helped us to achieve our goals. Please refer to the back of this Annual Report for a complete list of our generous supporters.

The Robertson Research Fund

The Robertson Research Fund continues to serve as a vital internal resource of support for our scientists. In 2008, it supported research in the labs of Grigori Enikolopov, Josh Huang, Leemor Joshua-Tor, Rob Martienssen, Senthil Muthuswamy, Bill Tansey, Anthony Zador, and Yi Zhong. Start-up research support was also provided by the Fund to four new investigators: Hiroyasu Furukawa, Raffaella Sordella, Lloyd Trotman, and Glenn Turner. In addition, the Robertson Research Fund continues to support the annual CSHL In-House Symposium and our programs for postdoctoral fellows and graduate students, the laboratory seminar program, and faculty recruitment.

Library and Archives

History of Biotechnology Meeting

While renovations progressed on schedule to the Carnegie Building, the home of the CSHL Library and Archives, there was also much progress in the expanded mission of this vital CSHL resource. Under the aegis of The Genentech Center for the History of Molecular Biology and Biotechnology at CSHL, Library and Archives, in cooperation with the Banbury meetings program that is run by Jan Witkowski, hosted a milestone meeting entitled “Biotechnology: Past, Present, and Future.” From September 21 to 23, scientists, entrepreneurs, and venture capitalists discussed the academic discoveries that gave rise to our modern biotechnology industry, and along with distinguished sociologists, economists, and historians, they discussed the importance of gathering and preserving primary documentary source materials that will enable historians of today and tomorrow to study the emergence of a field that has transformed the life sciences, business, and health care since its advent in the 1970s. I am pleased to note that our own Library and Archives department, under Ludmila Pollock, has already established a valuable repository for materials of lasting historical value, including an extensive and growing oral history archive.

Building Projects

Hillside Research Laboratory Campus

Progress on the construction of the six new research buildings of the Hillside Research Complex has been excellent. The Nancy and Frederick DeMatteis, David H. Koch, and William L. and Marjorie A. Matheson Laboratory buildings have progressed substantially toward their anticipated completion by the spring of 2009. The Donald E. Axinn, Leslie and Jean Quick, and Wendt Family Laboratory buildings are expected to be ready for occupancy by mid-2009. The landscaping surrounding the complex was significantly enhanced this year, and much-needed parking facilities have already been put to use.

The Hillside Research Laboratory Complex (*top*)

Access to the new complex is now possible via a roadway connecting to the Grace parking lot. As part of this project, we made the decision to reconfigure the lot for improved traffic flow and safety. Despite adding an additional traffic lane to better accommodate bus and van traffic associated with the Meetings and Courses program, we were able to increase green space in the area by more than 5000 square feet.

Renovation was also begun on teaching facilities in the Delbruck Laboratory. Structural problems necessitated reconstruction of the building's circa-1927 center section, requiring the entire top floor and roof section to be completely removed and rebuilt. Generous funding from HHMI allowed CSHL to completely modernize the entire historic teaching suite that is used for courses to train scientists on the latest technologies and techniques. These renovations and improvements are expected to be complete by the spring of 2009.

As noted above, we have made significant progress on the Carnegie Building renovation. Although we had originally hoped to complete the project by the end of 2008, design alterations and the challenges of working within an existing, historic structure made that impossible. The project is expected to be completed and ready for occupation in 2009.

We also undertook renovation and improvement of the circa-1937 Robertson House, located on the Banbury estate and primarily serving participants invited to high-level meetings at the Banbury Conference Center. Principal among the planned renovations was the replacement of the house's original electrical system in preparation for the installation of central air conditioning. The torrid weather of recent summers has made the house all but uninhabitable during the hottest part of the year. When completed in 2009, the house will have been completely freshened with new washrooms, refurnished, air-conditioned, and equipped with telephones and internet connections in each room. I thank Art Brings and Peter Stahl and all who worked on this difficult project to maintain Robertson House as a grand residence and doing so under budget.

CSHL has also continued its long-standing program of general campus improvements. The four-year project to upgrade and replace the building management system is now half

complete. The Jones Laboratory was renovated for course use during the Delbruck Laboratory renovations. Updates and changes were also made to various office areas to better suit the needs of the Laboratory's staff.

Special Events

National DNA Day

We celebrated the sixth annual congressionally designated National DNA Day on April 25, with walking tours of CSHL and festive signs on campus and throughout the village of Cold Spring Harbor. We thank the Cold Spring Harbor Library and Environmental Center and the Cold Spring Harbor Main Street Association for their participation.

Emerson String Quartet Concert

Cold Spring Harbor Laboratory, Stony Brook University, and Brookhaven National Laboratory hosted an evening of "Perfect Harmony" on April 1 to celebrate music, partnerships, and possibilities for increased research collaboration among the three scientific institutions on Long Island. Guests listened to the Emerson String Quartet and were treated to an appearance by pianist Gilbert Kalish.

Gavin Borden Visiting Fellows

The 14th Annual Gavin Borden Visiting Fellow Lecture, in memory of the publisher of *Molecular Biology of the Cell*, was held on April 14. The lecture was presented by Tania Baker, who in addition to being an alumna of the CSHL Undergraduate Research Program is the Edwin C. Whitehead Professor of Biology at the Massachusetts Institute of Technology and an HHMI Investigator.

The Lab Goes Latin

This year's *The Lab Goes Latin* event marked the 10th anniversary of the Cold Spring Harbor Laboratory Association's spring benefit. Held on May 10 at the Nature Conservancy in Cold Spring Harbor, it featured a packed dance floor with music by the urban salsa band Yerba Buena. The event raised more than $200,000 for CSHL's cancer and neuroscience research programs.

The urban salsa band Yerba Buena entertains at *The Lab Goes Latin.*

Symposium

The 73rd Symposium, on "Control and Regulation of Stem Cells," brought more then 300 researchers from around the world together at CSHL to discuss the latest findings in stem cell biology.

During the Symposium, the traditional Dorcas Cummings Memorial Lecture for scientists and guests from the community was delivered by Elaine Fuchs, Rebecca C. Lancefield Professor and head of the Laboratory of Mammalian Cell Biology and Development at The Rockefeller University.

Women's Partnership for Science

This year's event attracted 150 women from the surrounding community, New York City, and Connecticut to the home of Mr. and Mrs. Daniel P. Davison on June 22. They gathered to promote and support women pursuing careers in biomedical research. Guests participated in their own tabletop experiments on organic and genetically modified foods. The instructors were young educators from Cold Spring Harbor Laboratory's Dolan DNA Learning Center (DNALC); Erin McKechnie, a plant and soil specialist; and Elna Gottlieb, an earth science specialist.

Topping Ceremony

On July 22, CSHL celebrated the topping of the highest structure in the soon-to-be-opened Hillside Research Complex. Distinguished guests, elected officials, colleagues, friends, and members of Long Island's talented construction trades gathered to commemorate the occasion. They proudly observed as a 10-foot-6-inch square by 13-foot-high 5000-pound stainless steel pyramid was lifted by crane and secured to its new home atop an 85-foot tower that is called the Laurie and Leo Guthart Discovery Tower. In addition to the pyramid, a steel beam autographed by hundreds of CSHL staff and visitors is part of the foundation for the crown.

The Laurie and Leo Guthart Discovery Tower at the Hillside Research Complex

Paul Liam Harrison Exhibit

Paul Liam Harrison exhibited his artwork at CSHL during the Dynamic Organization of Nuclear Function meeting on September 16. For several years, his practice has become increasingly collaborative and has engaged with developments in and around the biosciences and, in particular, cell and gene research. The exhibit was a collection of works entitled "Designs for Life."

Harlem DNA Lab Opening

On September 23, New Yorkers celebrated the opening of the Harlem DNA Lab, a state-of-the-art education facility located in the John S. Roberts Educational Complex in East Harlem, New York. Joel I. Klein, New York City Department of Education Chancellor, and Peter Bruns, Vice President for Grants and Special Programs at HHMI, joined school principal Maria Aviles, DNALC Executive Director David Micklos, myself, and local leaders in opening remarks followed by a community tour of the teaching lab.

DNALC 20th Anniversary

On November 1, The Dolan DNA Learning Center celebrated the accomplishments of Executive Director David Micklos and his staff in transforming science education during the last two decades. The event included the dedication of the Laurie J. Landeau Multimedia Studio.

CSHL's Double Helix Medal

The Double Helix Medals Dinner

The Double Helix Medals Dinner was held on November 6 at the Mandarin Oriental, New York. Medals were presented to Drs. Marilyn and James Simons for corporate leadership, Sherry Lansing for humanitarianism, and James D. Watson, Ph.D. and J. Craig Venter, Ph.D. for scientific research. A total of $3.6 million was raised at the gala, which was cochaired by the Hon. and Mrs. Alan J. Blinken, Mr. and Mrs. Alan C. Greenberg, Dr. Arthur D. Levinson, Mr. and Mrs. David M. Rubenstein, Mr. and Mrs. Herbert J. Siegel, and Mr. and Mrs. Erwin P. Staller.

CSHL Public Lectures

January 21

Bruce Stillman, CSHL President: T*he Future of Molecular Medicine* at The Secret Science Club public science and art lecture series in Brooklyn, New York.

August 4

Scott Lowe, Professor and HHMI Investigator: *The Latest from the Frontlines of Cancer Research* at The Secret Science Club public science and art lecture series in Brooklyn, New York.

October 21

WLIW-TV "Healthy Minds" host Dr. Jeffrey Borenstein; Josh Huang, CSHL Professor; Jonathan Sebat, CSHL Assistant Professor; Linda Van Aelst, CSHL Professor; and Anthony Zador, CSHL Professor: A*utism: The Latest Genetic and Neuroscience Research and What it Means for Patients and Families*.

October 28

Jonathan Sebat, CSHL Assistant Professor and Anil K. Malhotra, Director, Psychiatry Research, Zucker Hillside Hospital and Associate Professor of Psychiatry, Albert Einstein College of Medicine: *Bipolar Disorder: Cracking the Code*.

November 11

CSHL trustee and poet Don Axinn: *Travel in My Borrowed Lives*.

CSHL Public Concerts

April 12

Jennifer and Angela Chun with Nelson Padgett, violins and piano

May 3

Soukhovetski and Vassily Primakov, piano duo

Harlem DNA Lab announcement

May 17
Anastasia Khitruk and Elizaveta Kopelman, violin and piano

August 30
Martin and Kristina Kasik, piano duo

September 20
Alexander Fiterstein and Steven Beck, clarinet and piano

September 27
Soyeon Lee, piano

October 4
Krista River and Judith Gordon, soprano and piano

Laboratory Employees

New Staff

Thanks to a superb recruiting effort led by CSHL Director of Research David L. Spector, we were pleased to introduce the following new faculty into the CSHL community this year.

Tom Gingeras, Ph.D., CSHL professor, is an established leader in the field of functional genomics who has developed high-throughput microarray technologies and powerful computation approaches to understand how genomes are organized and regulated. Before joining CSHL, Tom was Vice President for Biological Research at Affymetrix, Inc., California.

Assistant Professor Gurinder "Mickey" Atwal, Ph.D., is the first faculty appointment for the new Center for Quantitative Biology at CSHL. He will integrate computational, analytical, and experimentally derived data to approach several questions concerning the evolution and diversity of genomes. His projects include identifying changes in the genome that modify risk in cancer and autism; developing statistical tools for the analysis of interactions among genetic polymorphisms identified in large-scale genetic and epidemiological studies; and developing computational methods to detect networks of genes that have responded to evolutionary selection pressures.

Assistant Professor Bo Li, Ph.D., who completed his postdoctoral training at CSHL and at U.C. San Diego with Robert Malinow, studies neural synapses, specifically focusing on how synaptic dysfunction contributes to psychiatric disorders such as schizophrenia and depression. He uses a number of methodologies, including electrophysiology, genetics, and behavioral analyses, with a long-term goal of developing methods that allow for the manipulation of activity in specific brain circuits to correct disease-related behaviors.

Assistant Professor Zachary Lippman, Ph.D., studies the molecular mechanisms controlling reproductive fitness in plants. He uses genomic approaches to determine what controls flower, fruit, and seed production in tomato and *Arabidopsis*. His research will not only provide insights into plant evolution and domestication, but will also develop new tools for plant breeding. Zach joins us from the Faculty of Agriculture at Hebrew University of Jerusalem and is a Watson School graduate.

Associate Professor Pavel Olsten is both an M.D. and a Ph.D., whose research will provide a critical bridge connecting CSHL's genetics and neuroscience programs. He has developed a high-throughput approach involving cutting-edge imaging technology to monitor brain function at the level of synaptic circuits. He is studying how specific genetic mutations

and variations affect neural circuits in mouse models of schizophrenia and autism. Pavel was previously an Assistant Professor at Northwestern University.

Associate Professor Darryl Pappin, Ph.D., comes to CSHL to head the proteomics core facility on our campus. Proteomics is the large-scale study of proteins, and Darryl brings an impressive record of developing new methods for identifying and analyzing proteins in complex biological samples. Before joining us, he was a Scientific Fellow at Applied Biosystems, Applera Corporation.

Florin Albeanu, Ph.D., is a CSHL Fellow who received his doctorate from Harvard Medical School. Florin is an expert in imaging neuronal circuits in awake behaving rodents. Working in the olfactory system, he plans on using fiber-optic imaging and electrophysiological recordings to understand how neuronal circuits code information from the environment and how these circuits are shaped by sensory experience.

Ivan Iossifov, Ph.D., is a CSHL Fellow in Quantitative Biology from Columbia University. Ivan has devised computational methods to reliably extract knowledge about molecular interactions from the biomedical literature and combine this data with results from high-throughput biological experiments. In this way, he has built a framework to predict pathways or networks of interacting genes that contribute to common hereditary disorders. He is interested in applying his methods to improve conventional genetic analyses to detect correlations between specific mutations and common complex hereditary disorders such as schizophrenia, bipolar disorder, and autism.

CSHL Fellow Christopher Vakoc, M.D., Ph.D., is interested in how changes to the structure and organization of chromatin are related to cancer progression. He uses biochemical approaches to study specific modifications associated with leukemia and colon carcinoma. He did his doctoral training at Children's Hospital of Philadelphia.

Joining the administrative leadership of CSHL this year was Hans-Erik Aronson, Director of Information Technology. Hans-Erik was previously at the Center for Computational Biology and Bioinformatics at Columbia University. His own training in biochemistry and molecular biophysics, combined with his experience in design, implementation, and delivery of emerging technologies, will be critical to the success of individual scientific research projects as well as to the operations of the entire Laboratory, which increasingly depends on the strength of our information technology infrastructure and dedicated staff.

Promotions

Congratulations to Zhenyu Xuan, Ph.D., who was promoted this year to Assistant Research Professor and to Jim Hicks, Ph.D., who is now a CSHL Research Professor.

J. Hicks

Departures

During the course of the year, several faculty members took on new challenges at other institutions. Hollis T. Cline holds the position of Professor in both the Department of Cell Biology and Chemical Physiology at the Scripps Research Institute. Roberto Malinow is a Professor, Section of Neurobiology, and Professor, Neurosciences at UC San Diego. Zachary Mainen is Principal Investigator at the Champalimaud Foundation. Vivek Mittal is currently Associate Professor, Cardiothoracic Surgery, and Director, Lehman Brothers Lung Cancer Laboratory at Weill Cornell Medical Center.

Community Outreach

CSHL employees continue to actively participate in local and national community service events, including lab-wide blood drives in February, August, and December and the American Cancer Society's Daffodil Days in the spring. This year, CSHL employees donated 400 pounds of food to the Long Island Cares Harry Chapin Food Bank. Our campus also participated in the national campaign to donate used cell phones for conversion to 911 emergency-use cell phones that will be distributed to those in need by the Secure the Call Foundation. These efforts are organized by our dedicated Human Resources Department ably led by Katie Raftery. In addition to participating in local Long Island school activities, every year CSHL is proud to sponsor Cold Spring Harbor Library and Environmental Center kickoff festivities for the children's summer reading program.

CSHL campus walking tours were open to the public most Saturdays from March through November. Our specially trained team of tour guides, which includes CSHL graduate students and postdoctoral fellows, provides guests with scientific insights and personal perspectives that enrich the public's understanding of CSHL.

Looking Forward

The year 2008 was notable for the many accomplishments that I have highlighted here. The year was perhaps even more notable for the ability of this institution to manage its research and education programs in the face of significant external economic uncertainty. I credit the Laboratory's Principal Investigators, who are committed not only to scientific excellence but to the responsible management of their laboratory budgets and staff. The research administration team that we have built has succeeded in establishing a new standard for meshing the creativity required in scientific pursuit with the realities of fiscal and regulatory requirements.

CSHL's education programs have never been as strong and broad, reaching from middle and high school ages to undergraduates, graduates, postdoctoral students, and professional development for accomplished scientists. None of this would be feasible if not for the operational efficiencies and accomplishments of the Laboratory's administrative departments and their staff who provide critical support to our scientists and educators. In addition to those departments already mentioned, let me thank the entire Facilities Department, the Office of Sponored Programs, the Office of Technology Transfer, the Public Affairs Department, and the Purchasing Department for their great work. Despite a tough external environment, CSHL thrived as an institution and a community in 2008.

Thank you to our Trustees, faculty, staff, and many supporters for your hard work. As I look to the future, I remain confident that scientific research and education are a source of strength for the troubled national economy—a source that has yet to be tapped to its fullest potential. CSHL stands ready to push biomedical research forward to reach its fullest potential. With a sharp focus on our core mission, CSHL is poised to do even more for the future of science and society than we have in the many years of our very fruitful past.

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

CHIEF OPERATING OFFICER'S REPORT

We will forever remember 2008 as the year the bubble burst—driving the economy and the financial markets into chaos while leaving nonprofit institutions and universities around the world to cope with unprecedented depreciation of endowment funds. I am reminded of the somewhat tasteless joke, "Other than that, Mrs. Lincoln, how did you enjoy the show?" The analogy stems from the fact that 2008 was, in many respects, a very good year for Cold Spring Harbor Laboratory.

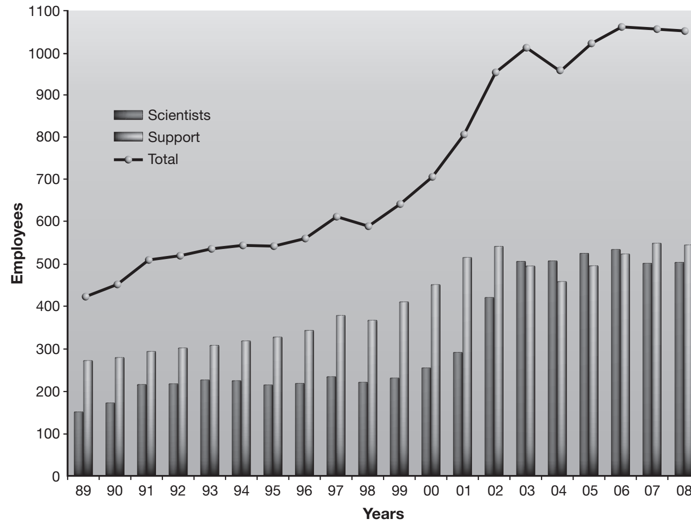
Construction of the Hillside Campus laboratory complex was near completion at year's end. The exteriors of the handsome 100,000 square foot, six-building research facility are finished, with final touches being applied to the interior spaces. The landscaping is nearly complete as well, with more than 500 new trees having been planted around the site. It is pleasing to be able to report that the project will come in within budget. Timing is everything, and we are fortunate that the project was in its final stages before the economic storm reached its full fury, allowing us to avoid the dilemma facing many universities with half-completed construction projects.

In 2006, we announced the ambitious plan to raise $200 million in support of the construction, faculty recruitment, start-up, and additional endowment. Again, we were fortunate to reach and surpass our goal before the massive loss of wealth in the public equity markets occurred late in the year. We are particularly grateful to our Trustees and the scores of friends who supported this campaign so generously, making it possible to reach this impressive milestone.

As you can see from the "President's Report," the Laboratory programs progressed in 2008 with unabated excellence. We saw high-profile research results and publications, well-attended meetings and courses, and the opening of a DNA Learning Center in Harlem. The Watson School continues to attract the best and brightest Ph.D. students to our campus and the Laboratory Press raises our institutional profile in the scientific community with its excellent list of journals, books, and online research protocols. The Carnegie Building, currently undergoing renovation, will be home to our expanding Library and Archives.

For the fiscal year ended 12/31/08, the Laboratory operating budget reached $123.5 million—an 8% increase over the prior year. Our objective each year is to balance the operating budget after full depreciation expense ($7 million in 2008) or, at a minimum, to be cash neutral after netting depreciation expense against cash expenditures on capital improvements. Fortunately, we were able to achieve this goal again this year. The primary contributing factors were (1) the success in unrestricted fund-raising via the Annual Fund, President's Council, and the Double Helix Medals dinner in New York City, (2) greater than expected private research grant income, and (3) vigorous efforts to manage overhead, administrative, and operating expenses. Much progress has been made during the last several years in the management of the grant process, research budgets, and general expenses, all to the benefit of the Laboratory's financial health.

Despite the progress and the fortunate timing on construction and fund-raising, we now face unprecedented financial and operating challenges as a direct result of the decline in the capital markets. In June of 2008, while our endowment had declined a relatively modest 3% year to date, we experienced a bump in the road that was an omen of things to come. The "auction rate securities" market, a $400 billion market for institutional paper, seized up and ultimately disappeared. The impact on the Laboratory was very real, because our 2006 $55 million bond issue consisted of this type of security. For a brief period of time, until we were able to convert the bonds to a different mode, the Laboratory was forced to pay extraordinarily high rates of interest in order to entice investors to buy our paper. This was ironic in view of the fact that the Laboratory's credit rating had recently been upgraded to a very healthy AA– status. The failure of the auction rate market was a precursor to the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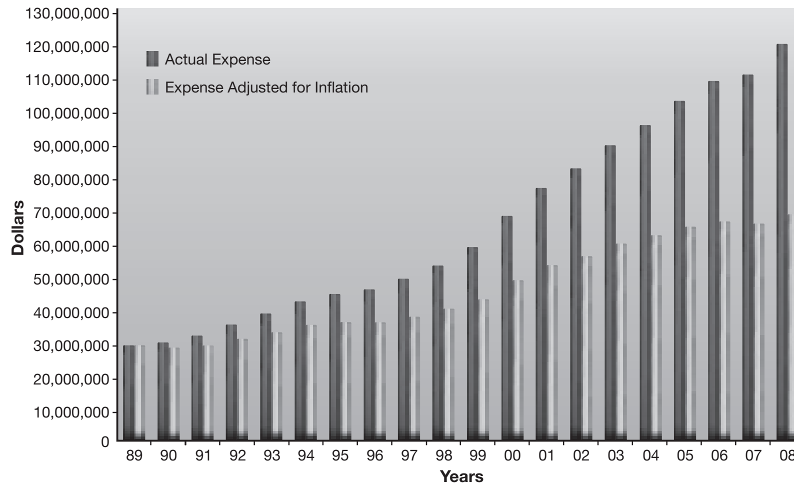
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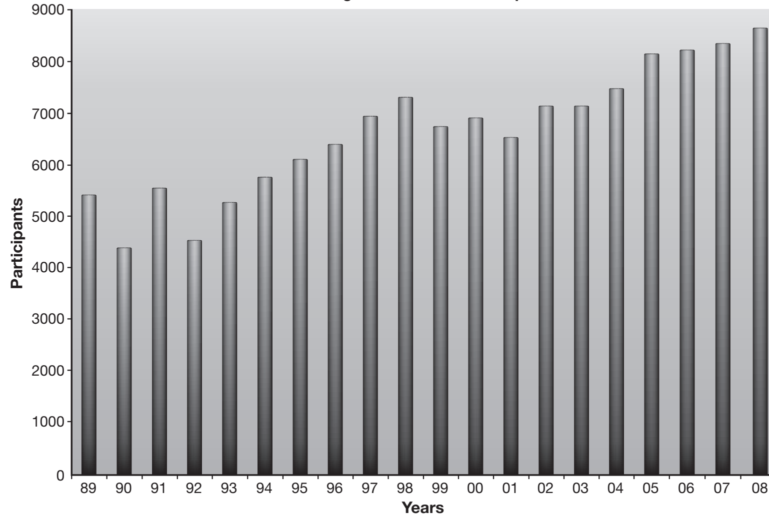
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general crisis in the credit markets, the extreme stress on the banking system, the precipitous decline in stock values, and the economic slowdown, all of which accelerated at an alarming rate during the second half of the year.

There was nowhere to hide for institutional endowments. The Laboratory endowment investment return was a negative 24% for our calendar fiscal year. Although certainly discouraging, it was not as bad as many, given a substantial allocation to fixed-income investments that held their value. Also on the positive side, we have maintained a very liquid investment portfolio and hold no “frozen” assets or private equity investments. Nevertheless, when we account for the spending on the fund, the market value has declined to approximately $220 million, down from its high of nearly $315 million only one and a half years ago. We also are party to an “interest rate swap” agreement that was entered into in 2006 to provide long-term protection against high interest rates on the Laboratory’s variable-rate long-term debt. As rates have dropped to historic lows, the “mark-to-market” valuation of this contract represents a liability to the Laboratory and has required us to post cash collateral with the bank counterparty until such time as rates move up and the liability decreases in value.

The dilemma now facing all institutions that rely on endowment funds is the budgetary impact these declines will have in the coming years. Like most universities, the Laboratory annually spends a percentage of the average market value of the endowment funds calculated over the most recent 12 calendar quarters. When we average in a year such as 2008 and confront the prospect of another down year ahead, we very quickly face big holes in the operating budget that are difficult to fill. Spending from the endowment represents about 12% of the Laboratory’s annual operating budget, much lower than most universities. On the other hand, private philanthropy represents approximately 25% of the budget and is a revenue stream that will certainly be under pressure given the state of the economy.

As we look ahead to 2009, Management will be forced to take prudent and tough measures. At the very least, administrative hiring will be curtailed and expenses, already having been substantially cut, will need to be further reduced. At a time when a great deal has been asked of a dedicated staff, we will undoubtedly be asking more. Austerity is not a pleasant prospect, but it is what the times demand.

Dill Ayres
Chief Operating Officer

LONG-TERM SERVICE

(*Back row*) Arne Stenlund, David J. Stewart, David Spector, James Watson, Bruce Stillman. (*Middle row*) Salvador Henriquez, Joan Ebert, Beatrice Toliver, Michael H. Wigler, Nancy Dawkins-Pisani, Christopher Mc Evoy, Louis Jagoda, Grigori N. Enikolopov. (*Seated*) Kim Gronachan, Patricia Maroney, Daniel Jusino, Margaret Falkowski, Ronnie Packer, Maryliz M. Dickerson.

The following employees celebrated milestone anniversaries in 2008:

40 years	James Watson
30 years	Patricia Maroney, Christopher Mc Evoy, Beatrice Toliver, Michael H. Wigler
25 years	Joan Ebert, Margaret Falkowski, Daniel Jusino
20 years	Margot Bennett, Francis T. Bowdren, Nancy Dawkins-Pisani, Maryliz M. Dickerson, Grigori N. Enikolopov, Jeffrey Goldblum, Kim Gronachan, Salvador Henriquez, Louis Jagoda, Ronnie Packer, Margaret Stellabotte, Arne Stenlund
15 years	Pamela Lancellotti, Wayne Pav, David J. Stewart

RESEARCH

See previous page for photos of the following scientific staff:

Row 1: T. Hige (Turner Lab); I. Hakker (Zhong Lab); B. Czech (Hannon Lab); E. Ghiban (McCombie Lab)
Row 2: S. Wu (Huang Lab); R. Zhao (Spector Lab); M. Monaco (Stein Lab); J.J. Han (Martienssen Lab)
Row 3: Y. Hua (Krainer Lab); G. Collins (Tansey Lab); K. Cook (Wigler Lab); A. Bhandari (Sebat Lab)
Row 4: A. Husbands (Timmermans Lab); S.D. Akshinthala (Muthuswamy Lab); M. Gierszewska, K. Hrecka (Skowronski Lab); Y.J. Yang (Hannon Lab)
Row 5: M. Feigin, V.A. Calleja (Muthuswamy Lab); V. Krizhanovsky (Lowe Lab); A. Reid (Zador Lab); A. Kepecs, D. Kvitsiani (Kepecs Lab)

CANCER: GENETICS

Cancer Genetics focuses on understanding the genetic basis of cancer, cancer progression, and development of resistance to chemotherapy.

Gregory Hannon is a pioneer in the study of RNA interference (RNAi) in mammalian systems. In RNAi, double-stranded RNA molecules induce gene silencing. Hannon is credited with the discovery of two enzymes, called Dicer and Slicer, critical in the RNAi machinery. (The work on Slicer was performed with Leemor Joshua-Tor.) His team has led the way in using RNAi to study cancer biology and genetics. They have generated a library of short-hairpin RNAs that researchers at CSHL and elsewhere apply broadly in gene-silencing studies. This year, the lab made a host of discoveries, including that of a new class of small RNAs, which was found in fruit flies to modify gene activity and suppress mobile genomic elements, thus serving as a defense mechanism. They also discovered 180 previously unrecognized microRNAs specific to monotremes in work stemming from a collaborative effort to sequence the platypus genome. The lab also explained a new way in which epigenetic information is inherited, discovering in fruit flies a class of small RNAs maternally inherited that determines an offspring's fertility trait.

Scott Lowe's laboratory studies cancer gene networks and determines how genetic lesions affecting these networks contribute to tumor development and resistance to therapy. This year, they continued to study cellular senescence, a potentially powerful mechanism for suppressing tumors, showing that it helps to limit wound-healing responses, a finding which suggests that it might act outside cancer to maintain tissue homeostasis following damage. Lowe also has adapted RNAi technology to produce animal models in which genes can be switched on and off in a spatial, temporal, and reversible manner and used this to identify and characterize new genes that modulate tumor cell responses to chemotherapy. He spearheaded an effort with other CSHL investigators to integrate genomic studies on human liver cancers with RNAi-based screening in a mouse model to identify 13 new tumor suppressor genes. These studies suggest a means of functionally annotating cancer genes and holds promise of producing new insights into the genetic basis of tumor diversity that can ultimately be exploited to tailor treatments to individual patients.

Robert Lucito, collaborating with Michael Wigler, has had an important role in developing innovative technologies, including representational difference analysis (RDA), representational oligonucleotide microarray analysis (ROMA), and comparative genome hybridization (CGH), that have proven to be valuable to cancer researchers worldwide. Also an experimentalist, Lucito has conducted studies using these techniques to detect copy-number changes in large sets of human ovarian and pancreatic cancer tissue samples. The lab also has turned its attention to epigenetics, specifically to the study of methylation throughout the genome. When methyl groups in sufficient numbers attach to cytosine bases in DNA, the packing of DNA into the chromosomes can be altered, reducing the levels at which the methylated genes are expressed. Under circumstances that Lucito is now exploring, this may be a means by which normally protective tumor suppressor genes are turned off, rendering cells tumorigenic.

Alea Mills is studying genetic pathways important in cancer and aging, identifying the genetic players and determining how aberrations in their functions culminate in human disease. Recently, through innovative use of a technique called "chromosome engineering," the Mills group identified a tumor suppressor gene that had eluded investigators for three decades. The tumor-suppressing gene, called *Chd5*, was shown by Mills to regulate an extensive cancer-preventing network. The epigenetic role of *Chd5* in development, cancer, and stem cell maintenance is currently being investigated. The Mills lab is also studying the p63 protein, which regulates development, tumorigenesis, cellular senescence, and aging, in vivo. Their progress during the last year indicates that cellular senescence has tumor-suppressing effects, a finding that indicates potentially important new avenues for anticancer therapies.

The discovery that stem-like cells involved in developmental decisions such as cell-fate determination can give rise to certain types of cancer has provided a new theme for cancer research. The identification of cancer stem cells with vital roles in tumor progression, maintenance, and recurrence has suggested

that at least some cancers arise from malfunctioning developmental programs. The research of CSHL fellow Patrick Paddison, which seeks to discover more about how molecular pathways promote or contain "stemness," may therefore provide insight into cancer biology and point to new therapeutic strategies. Using in vivo functional-genetics approaches, Paddison is attempting to identify genes required for self-renewal and lineage specification in embryonic and adult stem cells in humans and rodents. He seeks to determine if these genes are involved in stem-cell-driven cancers. His lab is also studying how to apply RNAi technologies to create cell types that one day might be used in cell replacement therapies.

RNA INTERFERENCE MECHANISMS AND APPLICATIONS

G. Hannon	A. Aravin	B. Czech	P. Guzzardo	I. Ibarra	A. Molaro	N. Shostak
	J. Brennecke	C. Dos Santos	A.D. Haase	F. Karginov	F. Rollins	V. Sotirova
	R. Burgess	Y. Erlich	X. He	M. Kudla	M. Rooks	O. Tam
	A. Canela	M. Evgenev	N. Hiramatsu	C. Malone	N. Rozhkov	V. Vagin
	K. Chang	K. Fejes Toth	I. Hotta	K. Marran	E. Rozhkova	E. Zelentsova
	S. Cheloufi	A. Gordon				

The Hannon lab continues to investigate the biology of small RNA and to apply small RNAs as tools to understand cancer. Through collaborations with the McCombie and Gingeras labs here at CSHL and other labs, we have expanded our efforts in genomics, particularly working to devise increasingly powerful ways to probe the cancer genome and to understand the diversity of small noncoding RNAs. These studies have required increasingly complex computational strategies, pushing efforts of the group toward the development of new tools for harnessing the power of next-generation sequencing.

The last year has seen many changes in the makeup of the lab. Julius Brennecke departed to assume a position as a group leader at the IMBA in Vienna, and Liz Murchison joined Jenny Graves and the Sanger Center as a postdoctoral fellow. Jose Silva joined the faculty of Columbia University. Monica Dus, Angélique Girard, and Despina Siolas each received their Ph.D.s. Monica left for a postdoctoral position with Greg Suh at New York University, and Despina returned to complete the medical school component of her M.D./Ph.D. program. Hannah Bender, a longtime visitor working on the Tasmanian Devil project, returned to Jenny Graves' lab.

We were joined by several new postdoctoral fellows. Andres Canela, who will continue to pursue his interests in cancer genome structure, came to us from the CNIO. Camila Dos Santos came from Mitch Weiss' lab, bringing her experience in hematopoiesis but changing her focus to mammary stem cell biology. Vasily Vagin joined us from Phil Zamore's lab where he had done fundamental work on small RNA pathways in the germ line. Nobu Hiramatsu came as a fellow from Japan to work on drug resistance mechanisms in lung cancer. Several graduate students joined the lab this year, namely, Paloma Guzzardo, Antoine Molaro, Ralph Burgess, Marek Kudla, and Ben Czech. Nik Rozhkov joined the fly group as a visiting student. Finally, Assaf Gordon joined our computational group, and he has worked tirelessly to make powerful informatics tools more accessible to the lab overall.

This year has seen many interesting stories emerge or initiate, and they are described in more detail below.

THE BIOLOGY OF SMALL RNAS

The Biology of Argonaute Proteins

S. Cheloufi

The genetic knockouts of Argonaute family members Ago2, Ago1, Ago3, and Ago4 demonstrate that Ago2 is the only Argonaute protein required for embryonic development. Ago2's unique role during embryogenesis could be explained by its specific association with small RNAs, its expression level and profile, or its specific catalytic activity. A set of independent point mutant alleles of Ago2 demonstrates that the catalytic activity of Ago2 is dispensable for embryonic development but is essential after birth. Preliminary evidence demonstrates that Ago2 does not associate with specific microRNAs (miRNAs) and that the expression pattern of Ago2 in the embryo is likely to be the key to its unique function during embryonic development. Inactivation of Ago2 catalytic activity rescues the early lethality of Ago2 past the organogenesis stage of embryonic development but is lethal a few hours after birth. The requirement of the catalytic activity of Ago2 at birth is still a mystery. We are currently investigating the requirement of Ago2 catalysis in lung development at birth. Preliminary data demonstrate that the point mutant lungs are immature and result in respiratory distress and cyanosis. This mouse model provides an essential tool for investigating cleaving small RNAs and their targets.

Small RNA-mediated Epigenetic Inheritance

C. Malone, J. Brennecke

Small RNA-directed silencing processes or RNA interference (RNAi) pathways participate vitally in chromatin regulation, repression of gene expression, viral defense, and silencing of selfish genetic elements.

At the core of each pathway acts an Argonaute protein, which in its active form is loaded with a single-stranded 21–30-nucleotide small RNA, guiding the

Argonaute protein complex to RNA targets with complementary nucleotide signatures. A variety of small-RNA-generating pathways act upstream of distinct Argonautes, resulting in the specialization of Argonaute effector complexes for the identity of their targets. We are interested in the in vivo functions of Argonaute proteins in *Drosophila*, especially those acting to protect the genome against selfish genetic elements such as transposons.

We have gained recent insight into the Piwi pathway, which seems to have specifically evolved to fight transposable elements, by investigating a remarkable phenomenon termed "hybrid dysgenesis." Here, crosses between two strains of *Drosophila melanogaster* produce sterile progeny. The reason for this is that one strain has gained control over a single transposon, whereas the other strain has not. We have now determined that *Drosophila* mothers epigenetically deposit Piwi protein complexes to offspring, imparting the ability to the progeny to effectively silence active transposable elements. Therefore, we have discovered that Piwi proteins and their bound small RNAs are the molecular basis underlying the phenomenon of hybrid dysgenesis.

A New Action of Penelope

N. Rozhkov, A. Aravin, R. Sachidanandam, E. Rozhkova, E. Zelentsova, N. Shostak, M. Evgenev

Three independent systems of hybrid dysgenesis are known in *D. melanogaster*; they are the P-M, I-R, and H-E systems. A fourth system is known in *Drosophila virilis*, where an unusual transposon, Penelope, has a key role and appears to be responsible for mobilization of the other five transposable elements. These elements belong to different transposon classes and unrelated families.

We have shown that Penelope in *D. virilis* is processed mainly into small interfering RNAs (siRNAs) (21 nucleotides) in contrast with other transposons that are targeted both by siRNA and Piwi-interacting RNA (piRNA) (23–29 nucleotides) pathways. It was therefore interesting to investigate how Penelope behaves in distant species such as *D. melanogaster*. These two species diverged from each other 40–60 million years ago. Penelope, injected in *D. melanogaster*, contributes only to the 21-nucleotide siRNA fraction. Thus, we suggest that *D. melanogaster* exposed to Penelope as a novel element can be used as a model system to study how the RNAi machinery adapts to invasion of new transposons or viruses.

Mechanisms of piRNA Biogenesis

A. Haase

We characterized two predicted nucleases involved in piRNA silencing. In addition to the Piwi proteins themselves, two putative nucleases have been linked to the piRNA pathway through genetic analyses. *Zucchini* (*Zuc*) encodes a protein with similarity to phospholipase D/nucleases (Koonin, *Trends Biochem. Sci. 21:* 242 [1996]; Ponting and Kerr, *Protein Sci. 5:* 914 [1996]), and *Squash* (*Squ*) shows limited similarity to RNase HII (Itaya, *Proc. Natl. Acad. Sci. 87:* 8587 [1990]). Mutants in these genes were initially isolated in a screen for female sterility. Recent studies have indicated that flies lacking either protein individually have some defects in piRNA-mediated transposon suppression (Schupbach and Wieschaus, *Genetics 129:* 1119 [1991]; Pane et al., *Dev. Cell 12:* 851 [2007]). We are evaluating *Squ* and *Zuc* as candidate piRNA-processing enzymes. First, we are characterizing small RNA populations in mutants by deep sequencing. (Heterozygote flies serve as control.) Char- acterization of small RNAs by deep sequencing has proven to be a powerful phenotyping tool for piRNA pathway mutants and has allowed assignment of many pathway components to germ-line versus somatic pathways and to the feed-forward piRNA amplification loop (C. Malone et al., submitted). Second, we are investigating the enzymatic activities of Zucchini and Squash using recombinant proteins in vitro. Third, flies expressing tagged transgenes are used to identify the respective ribonucleoproteins (RNPs) in the female and male germ line. Taken together, in vivo and in vitro analyses aim to elucidate the functions of these two predicted nucleases in piRNA biogenesis and to characterize their molecular mechanisms of action.

Common themes and differences exist in piRNA silencing in germ-line stem cells (GSCs) and their somatic niche. The expression of Aub and Ago3 is restricted to the GSC, where they are engaged in a feed-forward amplification loop of an initial piRNA signal, whereas in the male and female germ line, Piwi acts in GSCs and the surrounding somatic cells of the female germ cell niche and solely in somatic cells of the male germ line in *Drosophila*. We want to elucidate Piwi-only piRNA pathways in the somatic germ cell niche and investigate the relationship of Piwi to Aub and Ago3 in GSCs. To investigate Piwi pathways in GSCs and somatic cells of the GSC niche differentially, we have generated transgenic flies expressing a tagged Piwi protein under the control of the Gal4-UASp system, which allows tissue-specific expression of the transgene using different Gal4 drivers. (Alternatively, specific cell types

from germ-line tissues can be purified using biochemical fractionations.)

Overall, we aim to gain new insights into the molecular mechanism of transposon control and germ cell maintenance, as well as a deeper understanding of the functions and mechanisms of small RNA silencing pathways.

Proteomic Approaches to the piRNA Pathway

V. Vagin, A. Aravin

To better understand the mechanism of the production and function of piRNAs, we take advantage of transgenic animals expressing the FLAG-HA-tagged version of PIWIs. We purified all PIWI complexes from the fly and mouse germ lines. Very conservative proteins were identified in the mouse and fly PIWI complexes. Mutations in components of PIWI complexes lead to mouse and fly sterility, similar to mutations in PIWI genes.

Currently, we are analyzing the role of Tudor proteins in piRNA biogenesis in the mouse, using mutants for corresponding genes (in collaboration with Dr. Chuma, Kyoto University, and Dr. Wang, University of Pennsylvania). In parallel, we are studying the phenotype of the mutation in the *Tudor* gene in flies.

Genetic Approaches to Small RNA Pathways in Flies

I. Hotta

To identify components of the three Argonaute-dependent small RNA (siRNA, miRNA, and endogenous siRNA [endo-siRNA]) pathways in *Drosophila* S2 cells, we have undertaken a comparative and comprehensive genome-wide RNAi screen. Among candidates emerging from the screens, we identified subsets that act positively or negatively on siRNA, endo-siRNA, and miRNA pathways.

Endogenous siRNAs in *Drosophila*

B. Czech [in collaboration with R. Zhou, Harvard Medical School]

Small RNA pathways perform crucial functions in gene regulation, defense against viruses, transposon silencing, and formation of heterochromatin. The core effector complex of each small RNA pathway comprises an Argonaute protein associated with a single-stranded 19–30-nucleotide small RNA that guides the Argonaute protein to target transcripts sharing sequence complementarity. These small RNAs are categorized according to their mechanisms of biogenesis and the Argonaute protein to which they bind. Thus far, two classes of endogenous small RNAs have been identified in *D. melanogaster*: miRNAs, which associate with AGO1 and are required for proper development, and piRNAs, which act in concert with the Piwi clade proteins Piwi, Aub, and AGO3 to suppress mobile genetic elements. However, the endogenous binding partners of AGO2 have remained enigmatic.

Using biochemistry and deep sequencing of AGO2 immunoprecipitates, we showed that *Drosophila* generates a third endogenous small RNA class: endo-siRNAs. endo-siRNAs arise both from convergent transcription units and from structured genomic loci in a tissue-specific fashion and have the capacity to target virus transcripts, transposons, and protein-coding genes. We furthermore identified the double-stranded RNA (dsRNA)-binding protein Loquacious (Loqs)—thus far only known as an miRNA biogenesis factor—as being required for the biogenesis of endo-siRNAs derived from structured loci. Following up on our systematic analysis, we found that a specific isoform of Loqs, that is distinct from the one shown to facilitate processing or pre-miRNAs, is responsible for endo-siRNA processing. Deep-sequencing data and northern blots indicate that this specific isoform is also required for the production of endo-siRNAs derived from sources other than structured loci (e.g., endo-siRNAs targeting repeat sequences).

piRNA Clusters in Mammals

A. Aravin, A. Molaro

We investigated the biogenesis and function of piRNA in the mouse germ line. piRNAs are derived from extended genomic regions referred to as piRNA clusters. Using a recombineering approach, we inserted an artificial sequence (green fluorescent protein [GFP]) in two piRNA clusters. Modified piRNA clusters were introduced in the mouse genome as transgenes by pronuclei injection (in collaboration with Dr. Sang Yong Kim, Cold Spring Harbor Laboratory). Transgenic animals will be used to gain further insight into the mechanisms of piRNA biogenesis. We also investigated the function of piRNA in the establishment of de novo DNA methylation using piRNA reporter constructs where sequences complementary to abundant endogenous piRNAs were inserted in the promoter region of the gene expressed in the germ line. To test the association of the protein partner of piRNA, MIWI2 protein, with chromatin, we performed chromatin

immunoprecipitation (ChIP) analysis on sorted germ cells. Using small RNA cloning and high-throughput sequencing, we investigated the dynamics of piRNA populations during prenatal mouse development when de novo methylation patterns are established.

New Classes of Small RNAs

K. Fejes-Toth, V. Sotirova, A. Gordon [in collaboration with T. Gingeras, Cold Spring Harbor Laboratory]

The transcriptomes of eukaryotic cells are very complex. Noncoding RNAs dwarf the number of protein-coding genes and include classes that are well understood and classes whose nature, extent, and functional roles are obscure. We aimed to characterize the small RNA (<200 nucleotides) transcriptome of human cell lines. Deep sequencing of small RNAs from HeLa and HepG2 cells revealed a remarkable breadth of species that arose both from within annotated genes and from unannotated intergenic regions. Overall, small RNAs tended to align with CAGE tags that mark the 5′ ends of capped, long RNA transcripts. We have developed complex cloning strategies to characterize the biochemical properties of small RNAs and preferentially include or exclude species bearing different 5′ ends. These experiments revealed that many small RNAs, including the previously described promoter-associated small RNAs (PASRs), appeared to possess cap structures. An extensive class of both small RNAs and CAGE tags are distributed across internal exons of annotated protein-coding and -noncoding genes, sometimes crossing exon–exon junctions. These data indicate that processing of mature mRNAs through an as yet unknown mechanism may generate complex populations of both long and short RNAs whose apparently capped 5′ ends coincide.

Supplying synthetic PASRs corresponding to the c-*myc* transcriptional start site reduced *myc* mRNA abundance. Although some functional analyses indicate that at least PASRs do regulate the expression of the underlying coding genes, the possible regulatory function of these small RNAs needs further analysis.

SMALL RNAS AND CANCER

The miR-34 Family in Cancer

X. He

miR-34a lies in the p53 pathway and has been proposed to be a mediator of its tumor suppressive effects. We have generated constitutive knockout alleles of mir-34a in which the primary miRNA transcript is replaced by *lacZ* (the gene encoding β-galactosidase). The genotyping of targeted C57/BL6 embryonic stem (ES) cells has been accomplished by southern blot and polymerase chain reaction (PCR). Correct targeted ES cell clones were microinjected into the albino C57/BL6-derived blastocysts by the animal facility here at CSHL. The chimeric mice have been born and bred for germ-line transmission. Knockout mice have been recovered from the offspring, and the phenotypes of mir-34a-null mouse embryonic fibroblasts (MEFs) have been characterized. We have also targeted BL6/129 hybrid ES cells with the same constitutive knockout construct, and we are generating mice using the tetraploid–ES cell complementation method. In this approach, correct targeted ES cells were injected into 4*n* blastocysts, the ES cells gave rise to the epiblast, and the 4*n* host cells gave rise only to the placenta. This method allows us to study the consequences of mir-34a loss at an accelerated pace without the need for a chimeric intermediate. The correctly targeted ES cell clones were recovered at a rate of 30%, injected into 4*n* blastocysts by the animal facility, and the mir-34a-null mice have been identified. We are now studying the impact of miR-34a loss on p53 network function and tumorigenesis.

Roles of Argonaute and mRNA-binding Proteins in mRNA Control

F. Karginov, M. Kudla

Argonautes, the main components of the RNA-induced silencing complex (RISC), perform posttranscriptional regulation of most messenger RNA (mRNA). Guided by the imperfect complementarity of specific bound miRNAs to mRNAs, Agos cause translational repression or mRNA decay in a spatially and temporally defined manner, thus participating in many biological processes, such as differentiation, development, and disease. Although the core principles of Ago–miRNA–mRNA interactions have been established, many fundamental questions remain: What are the detailed rules of miRNA–mRNA interactions? How are these interactions regulated by other mRNA-binding proteins in various cellular processes, including stress response? What are the individual roles of the four mammalian Agos, particularly the catalytic activity of Ago2?

We are addressing these questions by a combination of biochemical and genomic techniques. In the past, we have developed a biochemical method to identify miRNA targets, and Ago-interacting mRNAs in general, by immunoprecipitation. Currently, we are using the method

to shed light on the role of Argonaute in translational regulation during the stress response. Furthermore, we are studying the role of the endonuclease activity of Ago by genome-wide 5′ RACE (rapid amplification of cDNA ends) approaches. In addition, we are constructing a transcriptome-wide library of 3′ UTRs (untranslated regions) to functionally interrogate their interactions with Argonautes and other mRNA-binding proteins. This tool will allow us to not only identify mRNAs that respond to particular miRNAs or mRNA-binding proteins, but also investigate their interactions on the mRNA.

Genetic Vulnerabilities of Human Cancers

K. Chang, K. Marran

We have shown that large-scale loss-of-function pooled screens using shRNA libraries can identify genes essential for survival or proliferation of human breast epithelial cells. Extending this approach during the past year, we have demonstrated the feasibility of using our shRNA libraries to perform genome-wide synthetic-lethal analysis for modifiers of drug (rapamycin) sensitivity in cancer cells. This analysis has enabled us to identify potentially new drug combinations with rapamycin by targeting new pathways that regulate phosphoinositol-3 kinase/mammalian target of rapamycin (PI3K/mTOR) signaling.

Furthering our screening capabilities, we have initiated a multi-institutional (CSHL; University of California, Los Angeles; Harvard Medical School; Baylor College of Medicine; Cancer Research UK; Fox Chase Cancer Institute; and the University of Michigan Comprehensive Cancer Center) collaborative effort to target breast cancer molecular subtypes using an integrative approach with the goal of yielding clinical impacts for improving the effectiveness of targeted breast cancer therapies. Our contribution to this cause is to perform genome-wide synthetic-lethal analysis for identifying critical targetable pathways in the three therapeutic categories: ER-positive, Her2-positive, and triple-negative disease. Our hope is to develop more rationally designed combination therapies that will eventually lead to new trials in the clinic implemented by translational members of our team.

Another area of application of RNAi screens is in the understanding of the molecular and pathological basis of pancreatic cancer. We and other laboratories here at CSHL are initiating a new collaborative program with the goal of developing new targeted therapies for this lethal disease.

Mechanisms of Tarceva Resistance

F. Rollins

This work has been in two major areas, both making use of the RNAi expertise of the Hannon lab. The first project is an RNAi screen using the Hannon-Elledge RNAi library to investigate modifiers of cellular response to targeted therapeutics. The current focus is on Tarceva (erlotinib; OSI Pharmaceuticals/Genentech), an FDA-approved agent for treatment of non-small-cell lung cancer and pancreatic cancer. The goal is to identify the genetic reasons underlying sensitivity to Tarceva and to make it a more powerful therapeutic. The second area of my research is to identify compounds for delivering RNAi molecules in vivo, which focuses on the use of different compounds to induce RNAi silencing of specific target genes, as well as carrier compounds used to deliver RNA molecules to cells in mice.

miRNAs and Breast Cancer

I. Ibarra, C. Dos Santos

Studies profiling miRNA expression in a heterogeneous murine mammary progenitor cell line (Comma-Dβ cells) by our group demonstrated that several miRNAs are differentially expressed in a self-renewing population (cells expressing ALDH-1), as compared to a more differentiated population. These findings led to the hypothesis that miRNA signatures provide a convenient strategy to classify and localize rare cell populations within a heterogeneous population and also suggested that manipulation of miRNA expression patterns might be useful for expanding or depleting stem cell and cancer stem cell populations, perhaps with therapeutic benefits. The classification of stem cells according to miRNA expression might demonstrate several advantages over traditional surface marker methods. Their ability to control the level of hundreds of different targets according to their presence, absence, or concentration represents a functional strategy to classify and mark cells according to their specification.

The stem-like mammary progenitor miRNA profile identified not only depletes miRNAs particular to this population, but also specific abundant miRNAs. These miRNAs could have an important role in the regulation of self-renewing properties, cell-stage maintenance, and differentiation. Therefore, manipulating the levels of abundant miRNAs present in mammary stem cells would also lead to a better understanding of the relationship between miRNAs and stem cells. Recently, cholesterol-conjugated single-stranded RNA complementary to miRNAs,

referred to as antagomirs, have being applied to silenced miRNAs. However, studies analyzing in vivo delivery of cholesterol-conjugated molecules suggested that significant amounts of cholesterol-conjugated antagomirs failed to immediately associate with lipoproteins and therefore cannot be transported to cells, suggesting that delivery of lipophilic conjugated molecules is efficient but not flawless. Attempting to find new approaches for an efficient antagomir delivery, we initiated a collaboration with Santaris Pharma, which developed a new generation of synthetic nonconjugated antago mirs, smaller than conventional antagomirs (~7 nucleotides). Theoretically, such small molecules should easily go through the cell membrane. Our preliminary results from in vitro and in vivo studies strongly suggest that a 7-mer antagomir is functional and demonstrates a potential role as a therapeutic agent.

NEXT-GENERATION SEQUENCING APPROACHES TO CANCER, DEVELOPMENT, AND EVOLUTION

Structural Variation in Human Cancer

A. Canela

The goal is to create an adeno-associated virus (AAV) vector for our shRNA library that will allow the use of shRNA and shRNA screenings in neuroscience. In collaboration with the Wigler lab here at CSHL, I am also evaluating the importance in breast cancer of genes involved in CNV (copy-number variations, characterized by the Wigler lab) by shRNA screenings and sequencing-by-synthesis technology.

We are characterizing structural variation in breast cancer genomes focusing on regions containing chromosomal rearrangements identified by comparative genomic hybridization (CGH) analysis (in collaboration with the Wigler lab). These regions are recovered from tumor genomes by array capture, and the precise structure of the genomic rearrangement is deciphered at single-base resolution by next-generation sequencing. These studies will provide the first deeply detailed picture of chromosomal alterations in breast cancers.

Analysis of DNA Methylation States during Development and Differentiation

A. Molaro, O. Tam, M. Rooks, E. Hodges

DNA methylation is an important marker of transcriptional regulation in the cell, and its presence correlates strongly with a repression of associated genes. This methylation pattern is dynamic, and it can be altered dramatically during the development and differentiation of the cell. Profiling of DNA methylation at various stages of development and differentiation could provide great insight into the cellular changes underlying these events.

The Hannon lab has recently developed techniques to analyze DNA methylation patterns throughout the genome, using a combination of microarray-based DNA enrichment and high-throughput deep sequencing. To enhance the capability of this technology, we investigated the possibility of working with lower amounts of DNA (as low as 1 ng), therefore allowing the study of low-abundance material (such as germ cells and developing embryos).

We have successfully generated bisulfite-converted DNA libraries from 2000 cells and are in the process of reducing the amount by tenfold. We have also designed microarrays that will enrich for genes and regions that have been implicated in stem cell maintenance, lineage specification, and repetitive element silencing, allowing us to analyze the methylation states at these loci. Our ultimate goal is to apply the technology to the study of DNA methylation patterns in preimplantation embryos, germ cell development, and hematopoietic differentiation.

Alta-Cyclic: A Self-optimizing Base Caller for Next-generation Sequencing

Y. Erlich [in collaboration with P. Mitra and D. McCombie, Cold Spring Harbor Laboratory]

Next-generation sequencers are revolutionizing biological research—they impact many aspects of genomics. Nevertheless, these sequencers are error-prone and suffer from shorter reading lengths as compared to conventional sequencers. We sought to improve the base-calling procedure for Illumina Genome Analyzers to obtain more accurate and longer sequence reads. Such an improvement would boost overall output per run, increase genomic coverage, and improve the ability to detect sequence variants. Longer reads also increase mapping precision and may even enable de novo genome assembly.

Inspired by ideas from communication theory, we analyzed the sequencing platform’s nonstationary distortion factors, because these accumulate throughout the run and reduce accuracy in later sequencing cycles. On the basis of our analyses, a model that describes two main signal distortions was created. The first distortion is due to chemical imperfections, dubbed as “phasing,” that induce heterogeneity in the nascent strand lengths across the DNA cluster. The heterogeneity reduces the purity of the signal from the interrogated position and

increases every cycle. The second distortion is an observed change in the spectral overlap between fluorophores as a function of cycle that induces a substantial bias toward certain base calls in later cycles.

We developed a novel base caller named Alta-Cyclic that is designed to specifically address these distortions. Alta-Cyclic works in two stages: the training stage and the base-calling stage. During the training stage, Alta-Cyclic learns run-specific noise patterns according to our model and finds an optimized solution that reduces the effect of these noise sources. The optimization is mainly achieved by supervised learning using a rich DNA library with a known reference genome. Alta-Cyclic then enters the base-calling stage and reports all of the sequences from the run with optimized parameters.

Benchmarking Alta-Cyclic with respect to the Illumina base caller after a long run of 78 cycles revealed a substantial increase in the number of correct base calls. For instance, in the case of a Phi-X library, Alta-Cyclic reported more than fourfold, 78-nucleotide-long, fully correct reads that comprised 22% of the total sequences. For comparison, the Illumina base caller reported only 5% of the total sequences without any misdetection.

Alta-Cyclic follows the open source philosophy for the benefit of the whole genomics community. The source code, installation, and usage instructions are available on our website: http://hannonlab.cshl.edu/Alta-Cyclic/main.html. The code is compatible with the current Illumina input and output formats and supports cluster computing using the Sun Grid Engine. The details of the mathematical modeling and the supervised learning algorithm are hidden from the users, allowing them to focus on the biological meaning of their data. In terms of development perspective, a great effort was made to write the base caller in a modular and scalable approach to allow future development by us or other users. In addition, every Alta-Cyclic installation constantly collects data regarding successful optimization parameters. The collected information is reported back to a central repository and will be used to enhance the training phase in later versions.

SUDOKU MULTIPLEXING: MASSIVE PARALLEL GENOTYPING WITH NEXT-GENERATION SEQUENCERS

DNA Sudoku: Indexed Applications of Next-generation Sequencing

Y. Erlich, K. Chang, M. Rooks

Next-generation sequencers have the potential to genotype in parallel DNA libraries that are composed of many different specimens—a practice known as multiplexing. A major requirement of a multiplexing scheme is the ability to associate each sequenced genotype with its original specimen. Current schemes rely on labeling each specimen uniquely using a short DNA tag, a process dubbed DNA bar coding. Unfortunately, unique bar coding of each specimen is expensive and labor-intensive; therefore, it is only practical for small-scale multiplexing experiments.

During the past year, we have developed a novel strategy called Sudoku Multiplexing. This strategy dramatically reduces the bar-coding procedures and expands the practical limits on multiplexing, realizing the sequencers' full potential. The power of Sudoku Multiplexing stems from encoding pools of specimens instead by assigning unique bar codes. To maintain the identity of the specimens, which may be lost in the pooling, the pools are constructed in a combinatorial way; each specimen is pooled several times, but each time, it is pooled with a different subset of specimens. We used a simple yet powerful theorem in number theory, called the Chinese Reminder Theorem, to create this combinatorial pooling design. The simplicity of the theorem allows for a straightforward translation of the encoding scheme to movements of a liquid-handling robot that carries the pooling procedure.

In the decoder side, the output of a multiplexed sequencing experiment is a set of combinatorial constraints, each of which corresponds to a different pool. A constraint can be thought of something such as "specimens #1, #6, #11, #16 can have sequences of type I, IV, V, VII." Indeed, a single constraint is not enough to decipher which sequence was derived from each specimen. However, an additional constraint such as "specimens #1, #7, #13, #19 can have sequences of type II, III, V, VI" automatically means that specimen #1 is associated with the sequence of type V. In addition, it reduces the uncertainty regarding the other specimen-sequence assignments. Thus, by combining constraints, one can eliminate the uncertainties and associate each specimen with its derived sequence. This situation is quite similar to the popular game Sudoku, where numbers are assigned to a grid using a set of constraints. However, both Sudoku and decoding the multiplexed sequences fall into the class of hard combinatorial optimization problems, where a tractable algorithm for any given input is unknown. We are currently developing a decoder that is based on a message-passing algorithm that has had substantial success in solving such optimization problems, even for quite difficult inputs. This algorithm combines the constraints in a soft way and gradually converges into the correct assignment combination (or close to it).

We demonstrated the power of our method by genotyping 40,000 bacterial colonies that comprise an shRNA library of 16,000 different fragments. The genotyping was carried by a single Solexa run and used only 384 bar codes and less than 2000 PCRs. Our method also has extensive implications for medical genetics, organ donor banks, prenatal screens, and population genetics studies.

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TUMOR-SUPPRESSOR NETWORKS

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	A. Chicas	S. Ebbesen	B. Ma	A. Rappaport	M. Taylor
	Y. Chien	C. Fellmann	K. McJunkin	M. Saborowski	W. Xue
	N. Dawkins-Pisani	C. He	C. Miething	C. Scuoppo	Z. Zhao
	K. Diggins-Lehet	V. Krizhanovsky	T. Pardee	J. Simon	J. Zuber

Cancer arises through an evolutionary process whereby normal cells acquire mutations that erode growth controls, leading to the inappropriate expansion of aberrantly proliferating cells. Such mutations can involve activation of oncogenes or inactivation of tumor-suppressor genes, each contributing one or more new capabilities to the developing cancer cell. However, cancer is not an inevitable consequence of oncogenic mutations; instead, cells acquiring such mutations can be eliminated or kept in check by innate tumor-suppressor programs that can be activated in these damaged cells. Our laboratory studies tumor-suppressor networks controlling apoptosis and senescence and how their disruption influences malignant behavior. We previously showed that apoptosis and cellular senescence are potent barriers to oncogene-driven tumorigenesis and that each contributes to the antitumor action of many chemotherapeutic drugs. Thus, not only do mutations that disrupt apoptosis and senescence promote tumor progression, but, depending on the particular lesion, they can also reduce the efficacy of cancer therapy.

To facilitate our research, we are combining advanced genetic and genomic tools that enable us to explore various aspects of cancer biology in a comprehensive way. For example, we have recently developed new mouse cancer models based on the genetic manipulation of stem and progenitor cells ex vivo followed by transplantation of the altered cells into the appropriate organ of syngeneic recipient mice. This approach allows us to rapidly study the impact of many genes and gene combinations on tumorigenesis in a “mosaic” setting where tumor-initiating cells are embedded in normal tissues. Furthermore, we have developed powerful methods for using RNA interference (RNAi) to suppress gene function in vivo in either a stable or reversible manner. Current efforts in the laboratory strive to integrate mosaic mouse models, RNAi, and cancer genomics to identify new components of tumor-suppressor gene networks and characterize their impact on tumorigenesis and treatment response. In addition, we are developing new RNAi methods to explore the role of tumor-suppressor genes in tumor maintenance and the cell death mechanisms involved in tumor regression.

Control of Cell Survival

C. Bialucha, K. Diggins-Lehet, C. Miething, C. Scuoppo, J. Simon [in collaboration with G. Hannon and A Krainer, Cold Spring Harbor Laboratory; J. Pelletier, McGill University; and former laboratory members H. Wendel, Memorial Sloan-Kettering Cancer Center, and A. Bric]

Normal cells possess intrinsic tumor-suppressor mechanisms that limit the consequences of aberrant proliferation. For example, deregulated expression of c-Myc or disruption of the retinoblastoma (Rb) pathway in normal cells can force aberrant S-phase entry and predispose cells to apoptotic cell death. Conversely, many oncogenic pathways promote cell survival by countering apoptosis. We have previously shown that oncogenes can engage the ARF-p53 tumor-suppressor pathway to promote apoptosis and that disruption of this pathway through loss of tumor suppressors or enforced expression of prosurvival genes cooperates with oncogenes to transform normal cells in vitro and promote tumorigenesis in vivo. We are currently interested in identifying additional components of these programs and understanding how they function in a tumor-suppressor network (see, e.g., Tworkowski et al. 2008).

Much of our current work on apoptosis and survival signaling exploits the Eμ-*myc* transgenic mouse model. This model is a model of B-cell lymphoma, where disruption of the ARF-p53 pathway, or overexpression of the prosurvival gene *Bcl-2*, cooperates to dramatically accelerate lymphomagenesis. More recently, we have used the Eμ-*myc* model to demonstrate that prosurvival signaling through the phosphoinositol-3 kinase (PI3K)/Akt pathway can substitute for *Bcl-2* in accelerating lymphomagenesis. During the past several years, we have been identifying components of this signaling network and studying whether they would serve as useful targets for therapeutic intervention. For example, we collaborated with Jerry Pelletier to identify a crucial role for the tuberosclerosis gene *TSC2* in suppressing Myc-induced lymphomagenesis (Mills et al. 2008). Moreover, we collaborated with former laboratory member H. Guido Wendel to implicate the TSC complex regulator Rheb as a potent human oncogene (Mavrakis et al. 2008) and also

with Adrian Krainer at CSHL to study how alterative splice factors modulate mammalian target of rapamycin (mTOR) activity (Karni et al. 2008).

Many of the downstream effects of the PI3K/Akt pathway appear to affect translation initiation. Indeed, we previously showed that the translation initiation factor *eIF4E* is a potent oncogene in vivo and can promote resistance to certain cancer therapies and later showed that its oncogenic activity correlates with its ability to activate translation and become phosphorylated on Ser-209. These results suggest that translational control of cell survival might be a therapeutic target and, indeed, our collaborator Jerry Pelletier used these model systems to test new inhibitors of translation initiation to show that they have antitumor activity in the Eμ-*myc* system (Bordeleau et al. 2008). Dr. Pelletier has recently joined the laboratory on a sabbatical, and future efforts will thus continue to examine the role of translation in survival signaling, in oncogenesis, and as a therapeutic target.

In a different approach, we have performed an RNAi-based screen (see below) to identify new tumor suppressors whose loss accelerates Myc-induced lymphomagenesis. One gene we identified as a potent tumor suppressor was *RAD17*, which acts to mediate DNA-damage responses to replicative stress. We showed that short hairpin RNAs (shRNAs) suppressing *RAD17* attenuate Myc-induced apoptosis and enable Myc-expressing cells to more rapidly proliferate. Further characterization of the mechanism by which *RAD17* suppression contributes to tumorigenesis is under way. We anticipate that other genes identified in this screen may also mediate apoptosis in response to Myc.

Roles and Regulation of Cellular Senescence

A. Chicas, Y. Chien, M.V. Krizhanovsky, J. Simon, W. Xue [in collaboration with Z. Yuan and M. Zhang, Cold Spring Harbor Laboratory; E. Hernando, New York University; and former laboratory members M. Narita, CRUK-Cambridge; L. Zender, Helmholtz-Gemeinschaft; and M. McCurrach]

Cellular senescence was originally described as a process that accompanies replicative exhaustion in cultured human fibroblasts and is characterized by a series of poorly understood markers. Senescent cells remain metabolically active, but they are unable to proliferate and display changes in gene expression that could alter tissue physiology. As such, they are genetically "dead" and cannot contribute to tumor development. Although "replicative" senescence is triggered by telomere attrition and can be prevented by telomerase, an identical end point can be produced acutely in response to activated oncogenes, DNA damage, oxidative stress, or suboptimal cell culture conditions. These observations have led us to propose that senescence acts in parallel to apoptosis as a cellular response to stress and acts in a similar way to suppress tumorigenesis and mediate responses to chemotherapy.

Our laboratory was the first to demonstrate that deregulated mitogenic oncogenes could drive cells into a senescent state thereby preventing transformation and that senescence could contribute to the outcome of chemotherapy in vivo. On the basis of the hypothesis that senescence is an important tumor-suppressive mechanism in vivo, we continue to study the roles and regulation of senescence. On the one hand, we have continued to explore how the p53 tumor-suppressor pathway activates a gene expression program to trigger senescence; on the other hand, we are interested in how the Rb tumor suppressor represses gene expression in senescent cells. Interestingly, regarding the latter, we are performing large-scale gene expression profiling experiments to identify Rb-regulated genes that are unique to the senescent state. These studies imply that Rb acts, primarily, to suppress genes involved in DNA replication, particularly in cells undergoing cell cycle exit into senescence. We believe that this may be crucial to its tumor-suppressive role and continue to work on the mechanism of this effect.

We also continue to be interested in the biology of senescence and its potential roles beyond cancer. Although senescent cells have been observed in some aged and damaged tissues, their functional contribution to non-cancer pathologies has not been examined. This year, we showed that senescent cells accumulate in murine livers treated to produce fibrosis, a precursor pathology to cirrhosis (Krizhanovsky et al. 2008; Krizhanovsky, in press). The senescent cells are derived primarily from activated hepatic stellate cells that initially proliferate in response to liver damage and produce much of the extracellular matrix deposited in the fibrotic scar. In mice lacking p53, a key senescence regulator, stellate cells continue to proliferate, leading to excessive liver fibrosis. Furthermore, senescent-activated stellate cells exhibit a gene expression profile consistent with cell cycle exit, reduced secretion of extracellular matrix components, enhanced secretion of extracellular matrix–degrading enzymes, and enhanced immune surveillance. Accordingly, natural killer cells, a major component of the innate immune system, preferentially kill senescent-activated stellate cells in vitro and are required to efficiently eliminate these cells in vivo, thereby facilitating the resolution of fibrosis.

The results described above suggest that the senescence program acts physiologically to limit the fibrogenic response to acute tissue damage and raise the possibility that it acts in other wound-healing responses as well. We

are also following up on work from 2007 showing that p53-deficient liver carcinoma cells could undergo senescence following p53 reactivation in vivo and that this program triggers an immune response that attacks the senescent tumor cells (Krizhanovsky, in press). Thus, we are continuing to explore the interplay between senescence and the immune system in the immune surveillance of developing tumor cells as well as in influencing tissue biology.

Mouse Models of Human Cancer

C. Bialucha, K. Diggins-Lehet, L. Dow, S. Ebbesen, L. Lintault, C. Miething, A. Rappaport, M. Saborowski, C. Scuoppo, J. Simon, M. Taylor, Z. Zhao, and J. Zuber [in collaboration with S. Kogan, University of California, San Francisco; C. Cordon-Cardo, Columbia University; and former laboratory member L. Zender, Helmholtz-Gemeinschaft]

Cancer is a heterogeneous disease that involves the accumulation of oncogenic mutations that each confers an advantage to the developing cancer cell. Moreover, it is now apparent that malignant tumors are not merely a collection of genetically identical cancer cells, but an organ that incorporates and is influenced by cellular and molecular components derived from normal tissue. As a consequence, the tissue microenvironment can have a dramatic effect on the tumor progression. Such issues place constraints on the utility of in vitro models to study all aspects of cancer development relevant to disease progression in patients. Therefore, to address these complexities, we have relied extensively on the generation and analysis of mouse models of human cancer.

Most mouse cancer models involve the generation of mouse strains that constitutively or conditionally harbor a cancer-promoting lesion in the germ line. The production of animals prone to the development of complex cancers typically involves the intercrossing of multiple cancer-prone strains that, although effective, can be time consuming and expensive. To address this issue, we have incorporated an additional approach that relies on the isolation of stem and progenitor cells from various murine tissues, their genetic manipulation in vitro using retrovirus-mediated transduction, followed by their retransplantation into syngeneic (immunocompetent) recipients. This approach is both flexible and rapid, because many combinations of cancer-predisposing lesions can be used to produce cancers in mice in a physiologic tissue compartment, without the time and expense of intercrossing many germ-line strains. Moreover, the resulting animals are "mosaics" in that the developing tumor cells are surrounded by a normal tissue microenvironment, much as would occur in human cancer progression.

Our initial use of "mosaic" mouse models involved modifications of the Eμ-*myc* lymphoma system described above, where hematopoietic stem and progenitor cells are isolated from bone marrow of fetal livers, manipulated in vitro, and then transplanted into lethally irradiated recipient mice. Indeed, we first used this system to characterize p53 effector functions relevant in lymphomagenesis and to document the utility of stable RNAi to mimic tumor-suppressor gene loss in vivo. More recently, we developed a mosaic model of hepatocellular carcinoma. Here, liver progenitor cells (hepatoblasts) are isolated, genetically modified in vitro, and transplanted into the livers of recipient animals. This system enabled us to explore the role of tumor suppressor gene loss in the maintenance of malignant progression and, in doing so, to identify a new form of immunosurveillance relevant to carcinoma biology. This year, we used these models in a number of important collaborations (Bordeleau et al. 2008; Gyrd-Hansen et al. 2008; Mills et al. 2008; Keng et al., in press; Laska et al., in press; Xu et al., in press; Ma et al., in press].

On the basis of the success of the mosaic approach, we have begun to develop additional mosaic mouse models to explore new aspects of cancer biology. For example, using procedures that parallel the Eμ-*myc* system described above, we have developed a series of mouse models of acute myeloid leukemia that harbor combinations of genetic alterations that occur in human patients. Interestingly, these models show general pathology and treatment responses as the corresponding human cancers (Zuber et al., in press), giving us confidence that these models can teach us about elements of cancer relevant to human patients. In addition, we are developing a mosaic model of breast cancer based on the isolation of mammary stem and progenitor cells and retransplantion of altered cells into cleared fat pads of recipient animals. As discussed below, we are using these models as tools for cancer gene discovery as well as for drug target identification and validation.

Integrated Approaches to Cancer Gene Discovery

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Because of its simplicity and ease of use, the mosaic mouse-modeling approach lends itself to rapid charac-

terization of cancer genes. We presume that most genes that modulate cancer in humans will do so in mice, and thus we have developed procedures that integrate genomic information from human tumors with mouse mosaic models to identify and characterize functionally relevant cancer genes. This effort was initiated in 2006, when we developed a system to rapidly clone and express functional shRNAs in mosaic mouse models. In the last year, we began to incorporate RNAi technology into our efforts to identify new cancer genes. Owing to its ability to suppress gene expression, we previously showed that RNAi serves as a rapid approach to mimic the consequences of tumor-suppressor gene loss.

In our first attempts to use RNAi for tumor-suppressor gene discovery, we used our mosaic model of hepatocellular carcinoma (HCC) to validate a tumor-suppressor gene on human chromosome 8p. Deletions on chromosome 8p are common in human tumors, suggesting that one or more tumor-suppressor genes reside in this region. One of these genes is deleted in liver cancer 1 (*DLC1*), which encodes a Rho-GTPase-activating protein. We showed that *DLC1* knockdown cooperates with Myc to promote HCC in mice and that reintroduction of wild-type *DLC1* into hepatoma cells with low *DLC1* levels suppresses tumor growth in situ (Xue et al. 2008). Cells with reduced *DLC1* protein contain increased GTP-bound RhoA, and enforced expression of a constitutively activated RhoA allele mimics *DLC1* loss in promoting hepatocellular carcinogenesis. Conversely, down-regulation of RhoA selectively inhibits tumor growth of hepatoma cells with disabled *DLC1*. Our data validate *DLC1* as a potent tumor-suppressor gene and suggest that its loss creates a dependence on the RhoA pathway that may be targeted therapeutically. They also illustrate the utility of stable RNAi to reveal new tumor-suppressor genes.

In addition to probing tumor-suppressor genes in vivo in a one-by-one manner, our laboratory (together with Drs. Hannon, Kraznitz, Hicks, Wigler, and Powers at CSHL) also performed in vivo screens to multiplex the analysis of candidate tumor-suppressor genes. In one example, we compiled pools of shRNAs targeting the mouse orthologs of genes recurrently deleted in a series of human HCCs and tested their ability to promote tumorigenesis in a mosaic mouse model (Zender et al. 2008). In contrast to randomly selected shRNA pools, many deletion-specific pools accelerated hepatocarcinogenesis in mice. Through further analysis, we identified and validated 13 tumor-suppressor genes, 12 of which had not been linked to cancer previously. One gene, *XPO4*, encodes a nuclear export protein whose substrate, EIF5A2, is amplified in human tumors, required for proliferation of *XPO4*-deficient tumor cells, and promotes HCC in mice. These results establish the feasibility of in vivo RNAi screens and illustrate how combining cancer genomics, RNAi, and mosaic mouse models can facilitate the functional annotation of the cancer genome.

In another example of this general approach, we performed an RNAi screen for new tumor-suppressor genes in the Eμ-*myc* mouse model described above. Pools of shRNAs targeting a focused set of cancer-relevant genes were introduced into hematopoietic progenitor cells derived from Eμ-*myc* transgenic mice and screened for their ability to promote lymphomagenesis following engraftment into syngeneic recipients. More than 20 candidate tumor-promoting shRNAs were isolated, including those targeting established tumor suppressors. Among the novel tumor suppressors were Rad17 (see above), whose reduced expression or loss is associated with lymphoma development and poor prognosis and therapy response in human patients. Some of these new tumor suppressors may have pro-oncogenic activities in other contexts, and a surprising number encode secreted proteins. The results of this screen are currently being finalized, and we have initiated a second-generation screen to extend our analyses.

Our results establish the feasibility of in vivo RNAi screens and illustrate how combining cancer genomics, RNAi, and mosaic mouse models can facilitate the functional annotation of the cancer genome. Most current efforts to catalog cancer genes rely solely on genomic approaches. Although they are powerful, genomic approaches can be expensive and yield candidates based only on statistical criteria. Virtually all candidates must be functionally validated in various in vitro or in vivo models, which is slow and likewise expensive. Through incorporation of our screening approach, it is possible to rapidly filter genomic information for genes that impact cancer development in vivo and thus focus follow-up studies on those that might be most clinically useful. Although our studies to date used mouse models of HCC and lymphoma and focused only on focal deletions, this relatively high-throughput approach could be expanded to other mouse models or include shRNA pools targeting genes affected by larger deletions, promoter methylation, or point mutations. Moreover, through exploitation of the emerging libraries of full-length cDNAs, it should be possible to perform parallel screens for oncogenes involved in genomic amplifications. Indeed, such efforts are currently under way. We believe that such integrative approaches will provide a cost-effective strategy for functional annotation of the cancer genome.

Molecular Genetics of Drug Sensitivity and Resistance

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Our laboratory has a long-standing interest in understanding genetic determinants that influence the cellular response to conventional chemotherapy, with the goal of identifying mechanisms of drug resistance and developing new drug targets. These efforts involve a combination of experiments using RNAi to characterize drug sensitivity and resistance genes, genomic approaches designed to identify genes that are linked to poor treatment responses in patients, and animal-modeling studies to test new drugs and drug combinations that might circumvent drug resistance.

Acute myeloid leukemia (AML) is an aggressive form of leukemia that displays a heterogeneous response to front-line chemotherapy, and many patients eventually acquire drug resistance. Owing to the tractability of AML models and the importance of the disease, we have had a long-standing interest in modeling the pathology and treatment sensitivity of this disease. To this end, we produced mosaic AML models that harbor common AML genotypes, established an induction chemotherapy protocol for mice, and integrated sensitive imaging methods to monitor leukemia response in vivo. We then used these tools to characterize genotype-response relationships in AML and to explore the underlying molecular basis for these effects. We see that a subtype of leukemia linked to poor treatment response in the clinic (harboring genetic translocations of the *MLL* gene) responds poorly to chemotherapy in mice and that this results, surprisingly, from an attenuated p53 response. Our studies provide insights into the differential response patterns of human leukemia, suggest strategies for improving the use of conventional chemotherapy, and produce tractable preclinical systems for testing new therapeutic strategies. Indeed, developing rational strategies to target these otherwise refractory leukemias is a major goal of current research.

To complement our mouse-modeling efforts, we have also initiated genomic studies to characterize genetic alterations associated with refractory or relapse AML. These studies are based on interactions with clinical colleagues at St. Jude Children's Hospital and the University of Chicago, as well as recently established interactions with Dr. Steven Allen at North Shore University Hospital on Long Island. We are using comparative genomic hybridization methods as well as next-generation sequencing technology to analyze leukemia specimens from patients that show poor responses to chemotherapy, and we intend to verify their ability to promote drug resistance to chemotherapy in mice. We hope to use this information to better inform the use of chemotherapy in the clinic and, eventually, to develop strategies to circumvent drug resistance.

Finally, we are integrating RNAi technology into our mouse-modeling efforts to identify and study factors that modulate sensitivity to cancer therapy. As a first step in this analysis, we performed a focused RNAi screen using the Eμ-*myc* lymphoma model to explore the genetic basis for heterogeneous responses to topoisomerase poisons (common chemotherapeutic agents used in the clinic). These experiments identified Top2A expression levels as a major determinant of the response to the topoisomerase 2 poison doxorubicin and showed that suppression of Top2A produces resistance to doxorubicin in vitro and in vivo. In addition, suppression of Top1 produces resistance to the topoisomerase 1 poison camptothecin, again leading to drug resistance in vivo. Unexpectedly, Top1 knockdown enhanced sensitivity to doxorubicin, leading to improved survival of tumor-bearing mice. These results highlight the potential utility of combining RNAi with mouse models to identify determinants of therapeutic outcome in vivo and form a blueprint for additional studies to uncover mechanisms of drug sensitivity and resistance.

Modulation of Gene Expression In Vivo Using RNAi

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Together with Gregory Hannon at CSHL, we continue to develop improvements in RNAi technology with a specific interest in using this approach to probe various aspects of cancer biology. We previously showed that pol II promoters expressing rationally designed primary microRNA (pri-miRNA)-based shRNAs ($shRNA^{mir}$) produce potent, stable, and regulatable gene knockdown in cultured cells and in animals, even when present at a single copy in the genome. As indicated above, we have used these tools to characterize tumor-suppressor genes, as well as to study the role of particular genes in the maintenance of malignant progression. During the past year, we continued to develop new methods for improv-

ing RNAi technology, particularly for applications in vivo. For example, we continue to develop improved vectors for expressing miRNA-based shRNAs in stable and conditional systems, thereby facilitating their use in genetic screens, and to study the role of particular genes in the maintenance of malignant progression. In addition, we have initiated studies with the Hannon group to develop shRNAs with optimal potency. These efforts, if successful, have the potential to revolutionize RNAi technology by enabling the production of genome-wide shRNA libraries with validated gene knockdown.

One of our most aggressive efforts of technology development is in the area of transgenic mice. We previously showed that it was possible to produce transgenic mice based on pronuclear injection of a linear fragment encoding an miR30-based shRNA downstream from the tet-responsive element (TRE). We initially developed transgenic mice based on a tet-responsive miR30-based p53 shRNA, crossed them to a variety of tet-*trans*-activator transgenic strains, and then showed that the presence or absence of doxycycline (a tetracycline analog) could reversibly knock down p53 expression in a tissue-specific manner. Now, we have produced a second generation of transgenic technology that rapidly generates fully transgenic founders with a defined genomic integration. In this generation of mice, the miR-shRNA is separated from the TRE by a green fluorescent protein (GFP) cDNA, conferring better shRNA processing as well as a simple marker for spatial and temporal induction of the TRE cassette. This improved system also obviates the need for any screening of different founder mice because the integration site is constant; together, these advances have drastically improved consistency, cost, and speed of generating inducible RNAi transgenic mice. We are currently developing a number of transgenic lines that conditionally express shRNAs capable of silencing tumor suppressors or potential drug targets. With improved tet-*trans*-activator lines, also under construction, we believe that this system can be used to spatially and temporally control the expression of any endogenous gene.

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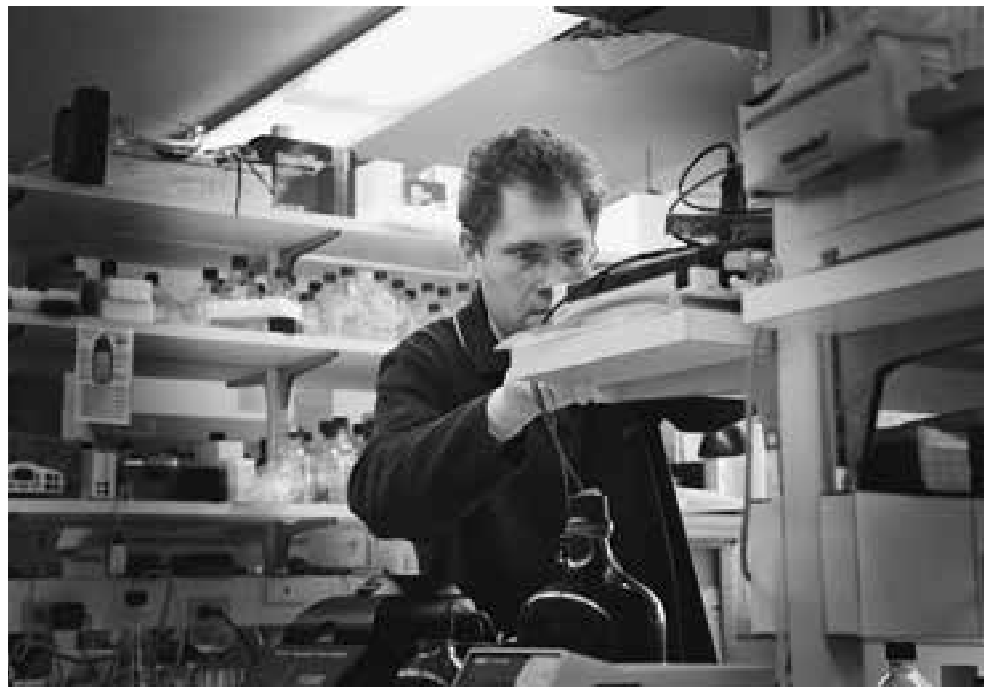

Valery Krizhanovsky

METHYLATION OLIGONUCLEOTIDE MICROARRAY ANALYSIS

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In addition to genetic mutation such as amplification and deletion, epigenetic aberrations are frequent events that can have far-reaching effects in the phenotype of a cancer cell. A common epigenetic modification is the methylation of cytosine residues that are next to guanine residues. It has been shown that methylation present in the DNA of the transcriptional control region has been involved in the silencing of gene expression of tumor supressors in cancer. We have adapted ROMA (representational oligonucleotide microarray analysis) to methylation detection oligonucleotide microarray analysis (MOMA). The present array queries all CpG islands within the genome. To detect methylation, input DNA (either tumor or normal) is cleaved with MspI and then ligated to polymerase chain reaction (PCR) adaptors and divided into two samples. One sample is digested with the endonuclease McrBC, which recognizes and cleaves methylated DNA, with the promiscuous recognition sequence A/GmC(N40–3000)A/GmC (Gast et al., *Biol. Chem. 378:* 975 [1997]) depleting methylated sequences. The other sample, the reference, is mock-digested. Both samples are amplified by PCR, differentially labeled, and hybridized to the array.

This methodology was used on cell lines, and 11 of 11 methylation measurements were validated by bisulfite sequencing confirming its accuracy. Although the standard method of methylation profiling of a tumor is to use matched normals, many tumor banks contain tumor samples without matched normals. Using a set of matched tumor normals (12 normals, 12 tumors) and a set of matched and unmatched tumor normals (12 normals, 28 tumors), we developed statistical criteria for identifying CpG islands most significantly altered between tumor and normal even when unmatched. We looked at all permutations comparing matched and unmatched tumors to all normals and found that all tumors generally varied similarly from the normals whether matched or unmatched. In other words, all tumors are similarly different from normal. We then moved onto the analysis of a larger set of 40 breast tumors, compared to 12 normal breast samples, and 11 ovarian tumors, compared to 7 normal ovarian samples. Using the statistical criteria developed, we obtained a list of 916 significant alterations in breast cancer and 151 in ovarian cancer, many of which have previously been documented to have altered methylation in cancer, again demonstrating the ability of this methodology to detect CpG methylation in cancer. For example, the *MTSS1* (metastasis suppressor 1) gene is known to be preferentially methylated in several cancers including breast cancer, and its lack of expression correlates well to increased Her2 expression. Genes that we identified as being hypermethylated in the ovarian and breast tumors include several HOX genes and protocadherins, which are known to be methylated in many tumor types. In addition to genes known to undergo methylation, we have found new targets of methylation. We also detected for the first time promoter methylation of a microRNA (miRNA) gene, *has-mir-9-3*, occurring in more than half of the breast tumors. The expression of this miRNA has been shown to be down-regulated transcriptionally in breast and other cancers.

Studies suggest that the methylation profile can be used to readily distinguish tumor samples from normal samples. To confirm and extend these observations, we performed hierarchical clustering on the 100 most significantly altered CpG islands between breast tumors and normals and ovarian tumors and normals. In all sample sets, the normals segregated from tumors. We also used a supervised machine-learning classifier to identify the sensitivity and specificity of using methylation to differentiate breast tumor samples from normals (the ovarian tumor data set was too small to perform this analysis) and used a "leave-one-out" cross-validation to calculate the sensitivity and specificity. Using only ten CpG islands, we achieved a classification accuracy of 94%. Sensitivity for tumor detection was 92.5% (37/40), and specificity for tumor detection was 100%, demonstrating that tissue can be identified potentially as tumor or normal based on a relatively small number of methylation alterations. In addition, a subset of the breast tumors have been shown to segregate into six subclasses based on expression analysis data, and hierarchical clustering performed on methylation data recapitulated these subclasses, demonstrating that methylation data can be used to stratify tumor samples. We propose that methylation data can be used to identify biomarkers that stratify with clinical parameters and will be studying this in the future.

We compared methylation status between promoter-specific islands (TSS-CGIs) and nonpromoter islands (non-TSS-CGIs), as well as nonisland CpG dinucleotides (non-CGIs), because this could be informative with respect to tumor evolution. We found that the methylation events that were frequently found were methylation of specific TSS-CGIs and non-TSS-CGIs and demethylation of non-CGIs. What was interesting was that for the class of non-TSS-CGIs, although we found some islands frequently methylated, the vast majority were demethylated. However, this demethylation was random, and rarely were the same islands demethylated in multiple tumors. This greater preponderance of demethylation in cancer without recurrence suggests that the demethylation of non-TSS-CGIs is more of a random event in the genome, compared to the methylation of TSS-CGIs being specific. Perhaps this is a result of the methylation of TSS-CGIs being positively selected during carcinogenesis in order to silence tumor suppressor genes, whereas the demethylation is not being selected for and the majority appears to be random. More than likely, methyltransferases are used for methylation, but it is unclear how demethylation occurs within an environment of methylation. It is possible that it is as simple as the methylation process on a whole being deregulated, resulting in some regions being aberrantly methylated and others not being methylated. Because we are looking at the tumor after its evolution, we only see the cells most successful at survival. The methylation of specific genes is selected for and the majority of the other methylation events are not observed because they are unselected. This will be the subject of future studies.

Methylome Analysis of Ovarian Cancer

C. Tang, S. Khan, S. Kamalakaran, J. Byrnes, N. Dimitrova

This year, in the United States alone, more than 25,500 women will be diagnosed with ovarian cancer, and more than 16,000 women will die of this disease. Five-year survival rates for women diagnosed with stage 1 or 2 ovarian cancer are 90% and 70%, respectively. Unfortunately, there is no reliable screening test for the early detection of ovarian cancer, and less than 35% of women are diagnosed before stage 3, with 5-year survival for stage 3 or 4 being less than 25%. Ovarian cancer is the leading cause of death from gynecologic cancer and the fifth most frequent cause of cancer-related death for women in the United States. Despite significant advances in surgical management and chemotherapy during the past few decades, the survival rate has not improved significantly. Currently, the 5-year survival for patients with advanced disease is less than 30%. A major impediment to successful treatment is the lack of therapeutic strategies that are effective against resistant tumors. Common and important genetic aberrations in ovarian carcinoma are poorly understood. Approximately 25% of patients will present with primary platinum-resistant disease and will have very poor outcomes. Additionally, all patients who recur will ultimately develop acquired platinum resistance.

We hypothesize that the tumors will have taken advantage of epigenetic mechanisms to become resistant to therapy and/or increase their ability to survive. Epigenetic modification such as CG dinucleotide methylation can have far-reaching effects on cellular phenotypes. We have used MOMA to analyze two sets of ovarian tumors—55 from Dr. Douglas Levine of Memorial Sloan-Kettering Cancer Center and 75 from Dr. Anne-Lise Borresen-Dale from the Norwegian Radium Hospital. Both samples sets have associated expression and copy-number data. Thus far, we have analyzed the methylation data and have begun the further analysis of identifying candidate regions that can segregate the tumors based on clinical variables such as platinum resistance. We have used clustering algorithms to analyze the data, and the normal samples clearly separate from the tumor samples. We can also see that the stage-2 tumors (tumors that have had less time to grow) have less methylation than the stage-3 and -4 tumors (tumors that have had more time to grow). This demonstrates that the tumors that have had more time to grow have had more time to accumulate alterations in the genome. Unfortunately, thus far, stages 3 and 4 could not be segregated further based on the methylation changes. We are continuing to analyze these data using different algorithms to identify either a pattern or specific events that can segregate the various stages and grades of the tumors.

We then analyzed the samples based on several clinical variables including survival and Carboplatin resistance. We identified islands that underwent methylation alteration and could segregate the samples based on the respective variable. We then compared the methylation data and expression data in a pairwise method to identify the genes whose promoters have undergone a methylation change and a corresponding expression alteration. We then used other methods including literature matching and pathway analysis to identify interesting candidates. These candidates will feed into two model systems that use short hairpin RNA (shRNA)

clones to mimic the transcription repression brought on by promoter methylation. The first will be a tissue culture system that will be used to test for genes that when methylated increase platinum resistance. Pools of shRNA clones will be introduced into cells, challenged with Carboplatin, and then allowed to grow. The cells that survive will be collected and the shRNA clones present will be identified. The second system, using a mouse model, will be to study genes that segregate with survival. Pools of shRNA clones will be introduced into immortalized mouse ovarian surface epithelial cells. These cells will be injected into a syngeneic mouse against the ovaries—a test pool on one ovary and a control on the other ovary. Growth characteristics will be monitored for the mice. These systems will identify the genes that when methylated affect the growth of the tumor and will address the critical problem of Carboplatin resistance in ovarian cancer.

Copy-number Analysis of Pancreatic Cancer

R. Lucito, S. Chen, J. Byrnes, N. Cutter

Of an estimated 30,000 cases of pancreatic cancer this year, 29,700 patients will succumb to the disease. Although the number of cases is low, compared 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 they seldom cure the patient. In fact, the mean survival time is ~6 months. Because the life span 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. Currently, we are collaborating with Dr. Ralph Hruban of Johns Hopkins, Dr. Daniel Von Hoff of the Arizona Cancer Center, and Dr. Vijay Yajnic of Massachusetts General, who will be providing pancreatic specimens, clinical information, and expertise. To date, we have analyzed 98 samples. We have confirmed many of the known mutated regions and have identified less-characterized regions.

One region on chromosome 19q12 that is amplified is interesting because there has been confusion in the literature as to which gene in the region is the oncogene in respect to pancreatic cancer. There are a number of tumors and cell lines that have this region amplified, but two primary tumors are the most informative for defining the epicenter or common region of mutation. We have previously shown that the gene p21-activated kinase or *PAK4* was overexpressed at the RNA level, over produced at the protein level, and overactivated as a kinase in cells with genomic amplification. This protein was also shown by others to transform cells when activated.

To elucidate the function of *PAK4* in the tumor, we have used shRNA constructs to knock down the level of *PAK4* in cells with the amplicon to determine the effect on tumorigenicity. After knocking down the level of *PAK4* in the cells, we collected RNA and performed expression analysis to determine whether there was a change in the cellular environment. Tumors are very heterogeneous, including their expression repertoire. Therefore, we performed this analysis for three separate cell lines with this region genomically amplified and used the results from all three to filter out noise and determine which were in common. Of the genes found with expression affected by *PAK4,* one interesting candidate is DUSP22 (JSP1), a phosphatase that is involved in selective activation of the mitogen-activated protein kinase (MAPK)/Jnk pathway, a pathway described below to be important for cell survival. Another is SMAD3, which is the binding partner of the tumor suppressor SAMD4, which is deleted or inactivated in ~50% of pancreatic tumors.

We have taken cells with *PAK4* shRNA that have activated K-*RAS* and have introduced these cells into nude mice; we found that the tumorigenicity of the cells is 40% lower with decreased *PAK4* activity. We have also determined that one function of *PAK4* is to increase the invasiveness of cells. Clearly, *PAK4* is required for some *RAS* ability to transform cells; however, *RAS* is complex. To better control the readout of the *RAS* activation, we have also added activated *PAK4* and activated K-*RAS* to NIH-3T3 cells and are comparing the cells transcriptionally to identify the genes *PAK4* activates that are in common with those genes that *RAS* activates. By performing these experiments, we hope to identify the role of *PAK4* in tumor cell transformation.

Mutational Analysis of Phosphatases

E. Lum, T. Auletta [in collaboration with N. Tonks, Cold Spring Harbor Laboratory]

The goal of this collaborative study is to integrate gene discovery technology with experimental strategies developed in Dr. Tonks' lab for the characterization of the protein tyrosine phosphatase (PTP) family of enzymes, to investigate how tyrosine phosphorylation-dependent sig-

naling pathways are disrupted in cancer. We have taken advantage of several different forms of genomic data to determine which PTPs are affected in cancer cells.

We first used genomic copy-number data and identified the PTPs, LAR, PTPσ and VHY amplified in ovarian tumor specimens. LAR and PTPσ a are receptor-like PTPs, display features of cell adhesion molecules, have been implicated in regulating the function of adhesion complexes that control cell–cell interactions, and may be disrupted in cancer. VHY is a dual-specificity phosphatase that the Tonks lab has implicated in the activation of a signaling pathway (the JNK pathway) that has a critical role in the control of cell survival. MKP5 is another dual-specificity phosphatase that functions in the inactivation of the p38 and JNK MAPKs.

We also used expression data to determine which PTPs undergo either up-regulation (putative oncogenes) or repression (putative tumor suppressors) in cancer. Expression data from ovarian cancer cell lines and primary tumors were used to determine if any of the PTPs described above found in regions of copy-number variation were aberrantly expressed. PTPσ and LAR showed the most significant expression changes, making them the best candidates. In addition to our analysis of PTPs that have undergone alterations with gene copy number, we conducted transcriptional expression analysis on the remaining phosphatases. Two sets of expression data were used to remove genes found in only one set; 15 phosphatases showed overexpression and/or underexpression in both sets. Of these, three overlapping phosphatases were found to show significant change, including DUSP6, PTPRK, and PTPRZ1.

Interestingly, a binding partner, Liprin, for the receptor PTP LAR, is highly amplified and displays altered gene expression. Liprin was shown originally to be important for targeting LAR to focal adhesions and for clustering the PTP at these sites regulating cytoskeletal function and interactions with extracellular matrix. This observation not only highlights the potential significance of LAR in ovarian cancer, but also, and more generally, introduces an interesting extension of the primary project, i.e., an analysis of the proteins that interact with the PTPs.

With the development of MOMA and the generation of methylation data for breast and ovarian cancer, we have recently identified PTPs that are methylated and by associating expression data have determined which are transcriptionally repressed. We have identified PTPN6 as a target in ovarian cancer. Interestingly, this PTP is an antagonist of VHY and suppresses the JNK pathway. We are currently looking to determine whether JNK is activated in cells with methylation of PTPN6. We have also expanded our extension of analysis of PTPs in cancer into the proteins with which PTPs interact by studying a gene whose promoter is methylated in breast cancer. This protein MTSS1 interacts with RTPδ, a receptor-like PTP that cooperates in cytoskeletal reorganization. RNA interference technology was used to show that when this gene was suppressed as found in the cancer cells, the cells gained invasive potential. The literature suggests that this gene may synergize with ERBB2 activation. Therefore, MTSS1 was suppressed by introduction of shRNA in cells that have an inducible ERBB2 pathway and the cells were grown in a three-dimensional culture. After activation, the cells grew faster and the growth restriction in three dimensions was lost, with the central cavity filling with cells and in many cases the acini overgrowing.

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MAMMALIAN FUNCTIONAL GENOMICS

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The Mills laboratory creates novel mouse models and uses them to elucidate the genetic and molecular basis of human disease. Research areas include (1) determining the role of the chromatin remodeler CHD5 in cancer and stem cell function and (2) investigating the role of the p53-related protein p63 in development, cancer, and aging.

CHD5 IS A TUMOR SUPPRESSOR MAPPING TO *1P36*

Deletions encompassing *1p36*, a portion of the genome residing on human chromosome 1, were first reported more than three decades ago. Since then, analyses of late-stage tumors have indicated that *1p36* is frequently deleted in a variety of human cancers, including those of the brain and blood and epithelial malignancies such as those of the breast, colon, and prostate. Although this had suggested that an important cancer-preventing gene maps to this locus, the gene responsible had not been identified. We recently identified *CHD5* as a novel tumor suppressor mapping to *1p36*. By using chromosome engineering to generate models with gain and loss of the region of the genome corresponding to human *1p36* and studying cancer predisposition or protection, we pinpointed a 4.3-Mb region of the genome with potent tumor suppressive activity. We discovered that whereas loss of this region led to a variety of cancers in vivo, gain of the same interval triggered excessive tumor suppression. Using a combination of genetic and molecular analyses, we identified the chromatin remodeling protein chromodomain, helicase, DNA-binding domain protein 5 (Chd5) as the tumor suppressor. We found that Chd5 functions as a master regulator that facilitates p16/Rb- and p19/p53-modulated pathways (Fig. 1). We are currently (1) assessing the spectrum of tumors that develop in mice heterozygous for the region encompassing *Chd5* and extending these findings to human cancers, (2) investigating the role of CHD5 in stem cells, and (3) determining the mechanism whereby Chd5 regulates chromatin dynamics. Thus far, we have identified a novel tumor suppressor. Our current studies aimed at elucidating the tumor suppressive role of Chd5 should pave the way for designing more effective anticancer therapies for a wide variety of human cancers.

FUNCTION OF THE *P53* HOMOLOG *P63*

p63 deficiency is tumor protective. The discovery that the *p53* tumor suppressor is a member of a multigene family that also includes *p63* and *p73* revolutionized the p53 field. p63 is a transcription factor that is structurally quite similar to p53; however, in contrast to p53, p63 is rarely inactivated in human cancers. We previously identified *p63* and generated a number of p63-deficient mouse models, which allowed us to determine that despite their similarities, p63 and p53 perform very dif-

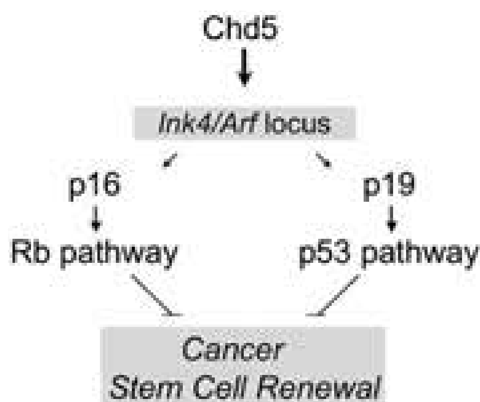

FIGURE 1 Chd5 is a master regulator of a tumor suppressive network. We propose that Chd5 induces chromatin remodeling events at the *Ink4/Arf* locus that facilitate expression of both p16 and p19. p16 and p19 are tumor suppressors that are responsible for enabling Rb- and p53-mediated tumor suppressive pathways. Thus, Chd5 functions as a circuit breaker that is necessary for this extensive tumor suppressive network to prevent cancer. p16 and p19 also regulate stem cell renewal, suggesting that Chd5 regulates the intimate relationship between stem cells and cancer.

ferent functions in vivo: p63 is essential for development of stratified epithelia, whereas p53 is dispensable during embryogenesis but functions as a potent tumor suppressor in the adult. More recently, we found that in contrast to the high incidence of spontaneous tumors in *p53*$^{+/-}$ mice, *p63*$^{+/-}$ mice were not predisposed to cancer. In fact, *p63* heterozygosity *decreased* the high tumor incidence of *p53*$^{+/-}$ mice, indicating that haploid levels of p63 may even be tumor protective. This indicated that p63 does not perform a role equivalent to that of p53 in tumor suppression and that reduced p63 provides a novel tumor suppressive mechanism. This finding has important clinical implications, because it suggests that modulation of p63-mediated pathways offers an effective strategy for anticancer therapy. We are currently working on determining which of the six different p63 proteins modulate the tumor suppressive mechanism of cellular senescence.

p63 links cellular senescence and aging. Over the course of the tumor study outlined above, we discovered that *p63*$^{+/-}$ mice had a significant reduction in life span and developed age-related pathology. By generating a new model that allowed us to turn p63 off specifically in proliferating cells of stratified epithelia such as the skin, we discovered an unanticipated link among p63, cellular senescence, and aging. Remarkably, p63 deficiency triggers cellular senescence and leads to aging in vivo. This suggested a mechanism for the low tumor incidence of *p63*$^{+/-}$ mice: Cellular senescence effectively removes aberrantly proliferating cells, thereby protecting from cancer. A further understanding of how p63-mediated pathways modulate stem cells and how this affects aging and tumorigenesis is currently under way. This work is expected to provide a clearer understanding of the mechanism of cellular senescence that will ultimately impact our understanding of tumorigenesis and stem cell homeostasis during aging.

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CANCER: GENE EXPRESSION AND PROLIFERATION

Gene Expression and Proliferation focuses on the regulation of gene expression, cell-division cycle control, and chromosome structure in normal and cancer cells.

The research of Thomas Gingeras and colleagues is adding significantly to our knowledge of where and how functional information is stored in genomes. This will help us to understand the biological and clinical effects of disease-causing gene mutations in humans and other organisms. Gingeras is a leader of the National Institutes of Heath ENCODE (*enc*yclopedia *o*f *D*NA *e*lements) project. His research has already revealed that nearly all of the human genome can be transcribed into RNA and that most RNA products are not made to be translated into proteins. Rather, "noncoding" RNAs are proving to be involved in a variety of other important biological functions. Some regulate protein-coding gene expression. Others serve as scaffolds upon which large protein complexes are assembled. The lab's research is helping scientists to significantly change long-standing notions about both genes and genomes, revisions so sweeping that they promise to change our very definition of the gene.

The laboratory of Leemor Joshua-Tor focuses on cell regulatory mechanisms, including protein complexes involved in nucleic acid regulatory processes. The lab uses X-ray crystallography to obtain three-dimensional structures of individual proteins and atomic-level views of their interactions with other molecules. This past year, they clarified how yeast cells, through the action of genes, adjust their metabolism in response to changes in their sources of food. This research suggests how the metabolic state of a cell is linked to the expression of its genes in a way that impacts biological processes of many kinds, ranging from cancer to aging. Joshua-Tor, who in 2008 was named a Howard Hughes Medical Institute Investigator, is well known for her work on the helicase enzyme, which acts to unwind DNA strands during the DNA self-replication process, and, in research with Gregory Hannon at CSHL, for revealing structures that help to explain the gene-silencing mechanisms of RNA interference.

Adrian Krainer and colleagues study the mechanisms of RNA splicing, the ways in which they go awry in disease, and the means by which faulty splicing can be corrected. Their approach has borne fruit in the study of spinal muscular atrophy (SMA), a genetic neuromuscular disease. Their ability to correct an mRNA splicing defect in SMA that makes a gene called *SMN2* only partially functional forms the basis of a potentially powerful therapeutic approach, now being tested in mouse models. They have also studied a splicing factor, SF2/ASF, that can cause cells to become cancerous and have now shown how it acts on proteins in a group called the PI3K-mTOR (phosphoinositol-3-kinase–mammalian target of rapamycin) pathway, known for its involvement in cancers. Measurement of SF2/ASF levels could eventually lead to identification of patients who will respond well to drugs that block the pathway. With Michael Zhang, Krainer this year also demonstrated a means of identifying the many genomic targets for a particular type of splicing factor and how splicing patterns of those targets change.

Scientists do not yet understand how dormant metastatic lesions, after they have colonized distant organs, grow into large lethal lesions. Vivek Mittal studies angiogenesis in the tumor microenvironment, which is composed of both malignant and nonmalignant cells. Among the latter are bone-marrow-derived endothelial progenitor cells, or EPCs, which, Mittal has demonstrated, become involved selectively in tumor blood vessel growth. This year, Mittal and colleagues, using mouse models, showed that the levels of a protein called Id-1 increase dramatically in EPCs when tumors are present. Using RNAi to block the expression of Id-1 in living mice, the team was able to prevent mobilization of EPCs to metastasis sites, thereby inhibiting an "angiogenic switch," a key mechanism that causes formation of blood vessels in tumors and triggers tumor growth.

David L. Spector's lab studies the spatial organization and regulation of gene expression. Their in vivo approach is exemplified in a live cell gene expression system that has made possible examination in real time of the recruitment of members of the gene expression and silencing machineries. A current research focus is the distribution of nuclear Polycomb proteins, known to keep genes in a silent state. The team seeks to target these proteins to segments of DNA as a means of selectively silencing spe-

cific genes. Another focus is the study of noncoding RNAs retained in the nucleus. This past year, the lab discovered a nuclear mechanism that processes noncoding RNAs. MALAT1, a noncoding nuclear RNA, was observed to split into two segments, the smaller of which migrated into the cytoplasm. A longer remnant remained in the nucleus, accumulating in zones called nuclear speckles. The nuclear-retained MALAT1 transcript is up-regulated in tumors that have the propensity to metastasize. Although it is not clear what the processed parts of the original RNA do, their disparate destinations in the cell suggest that they serve different functions.

Papillomaviruses, a large viral family that induces cell proliferation at the site of infection, usually give rise to benign tumors. But certain types of human papillomaviruses (HPVs) generate tumors that progress toward malignancy. Among these are HPVs that cause most cervical cancers. Arne Stenlund and colleagues have obtained a detailed understanding of processes required for initiation of DNA replication from the papillomavirus, using this system to gain a general biochemical understanding applicable in other systems. Members of the Stenlund lab also pursue studies aimed at developing an effective small-molecule inhibitor of HPVs that might someday be used by women who do not receive the preventive anti-HPV vaccine now available or those already infected with HPV who would not be helped by the vaccine.

Bruce Stillman's lab studies the process by which DNA is copied within cells before they divide in two. Working with yeast and human cells, Stillman and colleagues have identified many of the cellular proteins that function at the DNA replication fork during the S phase, the portion of the cell division cycle at which DNA synthesis occurs. Among these proteins are those that facilitate the assembly of chromatin, the protein–DNA complexes that form the chromosomes. The prime focus of current research, however, is the mechanism that initiates the entire process of DNA replication in eukaryotic cells. At the heart of this mechanism is a protein that binds to "start" sites on the chromosomes, called the ORC or origin recognition complex. Stillman's research has demonstrated that ORC is also involved in the process of segregating the duplicated chromosomes in mitosis. They have found ORC at centrosomes and centromeres, structures that orchestrate chromosome separation in mitosis.

Years ago, William Tansey observed that the destruction of transcription factors is intimately tied to their ability to activate transcription, which raised the intriguing question of how the very first step in the life of a protein was functionally related to the very last step, proteolysis—the protein's destruction by a molecular complex called the proteosome. The key link, he demonstrated, is ubiquitin, a molecule with which proteins must be tagged before they can enter the proteosome for destruction. Having linked ubiquitin and proteolysis to the activity of transcription factors, Tansey and colleagues have focused on the broader question of how ubiquitin affects transcription. This year, they discovered a means by which ubiquitin can regulate transcription without engaging the proteolytic machinery, specifically by acting as a wedge to pull apart and thereby inactivate RNA polymerase, the enzyme that orchestrates transcription. They are now exploring the biological context in which this nonproteolytic regulatory function of ubiquitin is brought to bear.

The function of DNA within cells is heavily influenced by its packaging with protein, a complex known as chromatin. The regulation of chromatin structure is essential for specifying which genes should be switched "on" or "off" in a given cell type. Alterations of chromatin structure that have been observed in cancer cells promote uncontrolled cell growth and progression of the disease. The research of CSHL Fellow Christopher Vakoc aims to understand how changes in chromatin contribute to the pathogenesis of acute leukemia, a cancer of blood cells. Using genome-wide mapping of chromatin structure together with a functional-genetics approach, Vakoc seeks to uncover novel chromatin pathways involved in cancer. By evaluating the reversibility of chromatin states in living organisms, he hopes to reveal novel therapeutic targets that may improve leukemia treatment.

ORGANIZATIONAL REGULATION OF EUKARYOTIC TRANSCRIPTIONS

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The gene has long been considered the basic functional unit of heredity in cells. The molecular structure of a gene has been fundamentally influenced by the model described by Jacob and Monod in their report (*J. Mol. Biol. 3:* 318 [1961]) on gene organization and regulation. Using this basic model of a gene, the organization and regulation of genomes have been studied and elaborated upon by several generations of molecular biologists. However, the advent of more genome-wide investigations made possible by means of novel experimental (e.g., second-generation sequencing and high-density arrays) and computational methods has provided a much more complex and elegant vision of the organization and regulation of eukaryotic genes and genomes. Our studies have centered on this emerging new organization and regulation as well as epigenetic influences on gene expression. We have sought a better definition of the basic unit of genetic information and to understand the gene in this new context. A first step to achieve a better understanding is to determine a complete parts list and genomic location of the functional elements in genomes. Our efforts toward this end have been to catalog the sites of transcription, characterize the RNA products made at these locations, determine the regulatory sites controlling these transcriptional products, and determine the function and fates of the non-protein-coding transcripts made by genomes. Results from these and other similar studies are having a significant influence on our understanding of how perturbations in DNA structure and RNA processing lead to many of our complex disease conditions.

TRANSCRIPTOME OF HUMAN CELLS

The NHGRI-sponsored *Enc*yclopedia *o*f *D*NA *E*lements (ENCODE) project has focused during the last 6 years on identifying and cataloging the functional elements found in the human genome. Our group has been a member of this consortium since its inception in 2002. One of the key functional elements to be identified and characterized is the transcriptomes of human cells. Investigators from seven international institutions—Gregory Hannon (CSHL), Yoshihide Hayashizaki (co-PI) and Piero Carninci (co-PI) (RIKEN), Yijun Ruan (co-PI) and Chia Lin Wei (co-PI) (Genome Institute of Singapore), Stylianos Antonarakis (co-PI) (University of Geneva) and Alexandre Reymond (co-PI) (University of Lausanne), and Roderic Guigo (Center for Genomic Regulation)—are collaborating with our group to generate a comprehensive set of subcellular compartment-specific long (>200 nucleotides) and short (<200 nucleotides) polyadenylated (poly[A]$^+$) and nonpolyadenylated (poly[A]$^-$) RNA maps for each of the two cell lines (K562 and GM12878) and for several primary and transformed cell lines in succeeding years. Long and short RNA (poly[A]$^+$ and poly[A]$^-$) maps from six subcellular compartments (nucleus, nuclear matrix, nucleolus, chromatin, cytoplasm, and polysomes) have been constructed. Additionally, the 5′ and 3′ termini of these transcripts have been identified by construction of both CAP analysis of gene expression (CAGE) and paired-end diTAG (PET) maps. (A path to all maps and raw and processed data can be obtained from http://www.genome. gov/10005107.) Finally, with the use of a combination of 5′ and 3′ rapid amplification of cDNA ends (RACE) reactions, reverse transcriptase–polymerase chain reaction (RT-PCR), and cloning and sequencing, the full-length structures of several thousand compartment-specific noncoding RNAs are being determined to assist in determining their possible function.

TRANSCRIPTOMES OF FLY CELLS

The NHGRI-sponsored model genomes (mod)ENCODE project has focused during the last 2 years on identifying and cataloging the functional elements found in the fly and worm genomes. Our group is a member of a consortium of five laboratories—P. Cherbas/L. Cherbas (Indiana University), S. Celniker (Lawrence Berkeley National Laboratory), M. Brent (Washington University), B. Gravely (University of Connecticut), and N. Perrimon (Harvard University)—focused on ultimately characterizing the transcriptomes of *Drosophila melanogaster* during each of 30 time points of development in 50 cell lines and in 30 diverse tissues.

During this year, 34 RNA samples (5 cell lines and

29 developmental time points, all in biological triplicate) were processed using 38-bp resolution whole-genome tiling arrays, and 34 RNA expression maps were generated. Our progress, including status of the RNA samples (total and poly[A]$^+$ RNA) and quality control data for individual samples, can be found at http://transcriptome.affymetrix.com/download/modENCODE/sampleQC/. Furthermore, transcribed regions (transfrags) were identified using three different thresholds to analyze tiling array data generated by our laboratory. These data, which essentially complete the proposed developmental time course, have been submitted to the modENCODE data coordination committee (DCC).

Using the microarray data, the Cherbas group has cataloged novel transfrags by sample and novel transfrag contigs (working over all RNA samples). We have assigned expression scores to all previously annotated exons and to the novel transfrag contigs. We have supplemented the microarray data by obtaining a Nimblegen whole-genome tiling array for comparison of platforms. We have also set up and are operating a Wiki for all of the Celniker group expression data. Our analysis of data from more than 25 cell lines points to the existence of ~86,000 novel transfrag contigs covering 28 Mbp (23% of the euchromatin). Analytical efforts relating microarray data to RNA-seq data including paired-end RNA-seq data are ongoing.

This year, we also generated two new updates of the transcript annotations for a total of six versions. The latest, MB6, comprises 21,504 transcript models at 12,789 loci and used expressed sequence tags (ESTs), cDNAs, and comparative genomic data. We performed quarterly cycles of cDNA clustering, N-SCAN-EST predictions, RT-PCR splice junction validation experiments, and incorporation of new ESTs. In addition, we improved our algorithms for building models to identify and track polycistronic genes.

To annotate overlapping genes on opposite strands, we modified N-SCAN to predict on each strand independently, rather than both at once. This approach reduced accuracy too much, so we abandoned it. However, overlapping genes are now annotated because we have integrated FlyBase annotations into our pipeline as hypothesized gene structures, on a par with N-SCAN predictions. They are then classified according to their level of support (see below).

We now have scripts that submit each new annotation almost automatically. We created Wiki pages showing the details of our protocols. We use GBrowse (http://mblab.wustl.edu/cgi-bin/gbrowse/fly5) to visualize and validate preliminary data that are not yet ready for submission to the DCC.

We implemented a system for classifying transcript models according to their level of supporting EST and cDNA data (one of our key deliverables) that will be displayed as a color code on the main modENCODE browser. There are nine levels of confirmation, of which the top three are as follows.

- Level 1 (11,133 transcripts): A full-insert cDNA has all splice sites in common with the model (neither has a site not contained in the other).
- Level 2 (3481 transcripts): A single assembly of cDNA sequences (including ESTs) has all splice sites in common with the model.
- Level 3 (2426 transcripts): Multiple, nonoverlapping assemblies of cDNA sequences confirm all splice sites, but there may be gaps in the coverage of exons. These may include incorrectly split or merged genes.

The remaining six levels describe models with one or more unconfirmed splice sites and single-exon genes. We also changed our pipeline to make it easier to compare our annotations with those provided by FlyBase. One of the major challenges and opportunities of the year has been the emergence of RNA-seq as primary data. This required a refocusing of effort that is now starting to pay off through validation of novel splice sites.

POSTTRANSCRIPTIONAL PROCESSING OF RNAs INTO SHORT, CAPPED RNAs

The results from our laboratory and those from many others have indicated that the number of individual noncoding RNAs greatly exceeds the number of protein-coding transcripts. The functional roles of most of these noncoding transcripts are still unclear. Two years ago, we proposed that the fate of a large proportion of the detected long noncoding RNA transcripts was to be processed into short functional RNAs (Kapranov et al., *Science 316:* 1484 [2007]). One collection of novel short RNA candidates that are likely derived from long RNA precursors was found flanking both sides of transcriptional initiation sites (TSSs). These we called promoter-associated short RNAs (PASRs). In collaboration with the Hannon laboratory here at CSHL, we analyzed this and other classes of short RNAs found in two human cell lines (HeLa and HepG2). Deep sequencing of small RNAs (<200 nucleotides) from these two cell lines revealed a remarkable breadth of short RNA species. Short RNAs were found to arise both from within annotated genes and from unannotated intergenic regions. Overall, small RNAs tended to align with CAGE tags,

which mark the 5′ ends of capped, long RNA transcripts. Many small RNAs, including the previously described PASRs, also appeared to possess cap structures as part of their structures. An extensive class of both small RNAs and CAGE tags were distributed across internal exons of annotated protein-coding and noncoding genes, sometimes crossing exon–exon junctions. These data indicated that processing of mature mRNAs through an as yet unknown mechanism is likely to generate complex populations of both long and short RNAs whose apparently capped 5′ ends coincide (Fig. 1). Supplying synthetic PASRs corresponding to the c-*myc* transcriptional start site reduced *myc* mRNA abundance.

GLOBAL TRANSCRIPTIONAL ACTIVITY IN PLURIPOTENT EMBRYONIC STEM CELLS

Embryonic stem (ES) cells are unique in their capacities to self-renew and to initiate differentiation into any cell type of the three germ layers. These opposing abilities are in part brought about by the presence of stem-cell-specific proteins (Takahashi and Yamanaka, *Cell 126:* 663 [2006]). During differentiation, lineage-specific transcription factors activate the expression of specific sets of genes that are required for each specific lineage. Such hierarchical activation is consistent with genetic transcriptional networks that operate during differentiation and development (Szutorisz and Dillon, *Bioessays 27:* 1286 [2005]).

In addition to control by specific transcription factors, regulation via epigenetic mechanisms has recently emerged as a key mechanism in pluripotency and lineage. Embryonic stem (ES) cell chromatin is characterized by several specific features that distinguish it from that of somatic and differentiated cells. In ES cells, heterochromatin is organized in larger and fewer domains, which, as cells differentiate, become smaller, more abundant, and hypercondensed (Meshorer and Misteli, *Nat. Rev. Mol. Cell. Biol. 7:* 540 [2006]). Another striking feature of stem cell chromatin is the altered binding of structural architectural chromatin proteins. The heterochromatin component HP1, the linker histone H1, and core histones all display hyperdynamic and looser binding to chromatin in undifferentiated ES cells than in differentiated cells. Interestingly, this hyperdynamic plasticity was exclusively found in pluripotent cell types, although not in lineage-committed but undifferentiated cell types, indicating that dynamic chromatin plasticity is associated with pluripotency rather than differentiation per se.

ES cells also contain unique histone modification patterns. Extensive regions of the genome are bivalently marked by transcriptionally repressive H3-triMeK27

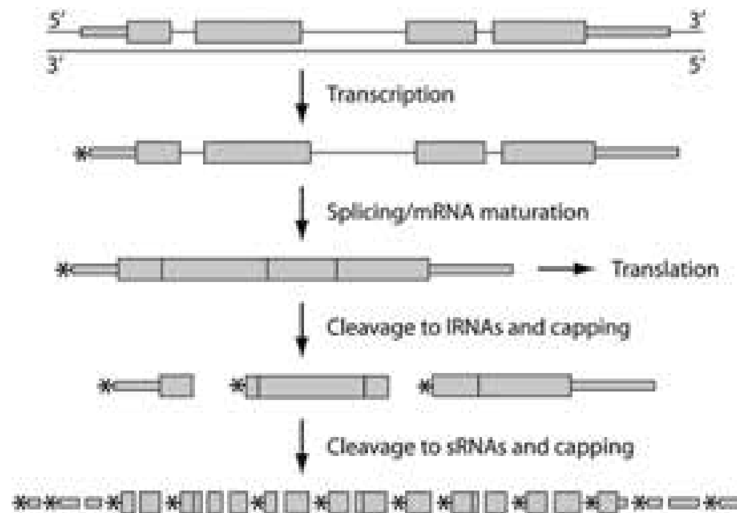

FIGURE 1 A proposed model for the metabolism of genic transcripts into a diversity of long and short RNAs. Transcription of a genic region results in a precursor long RNA containing a 5′ cap structure. After processing into spliced RNAs, protein-coding RNAs are either destined to be translated or to be further processed. Further processing entails cleavage followed in some cases by addition of a 5′ modification, possibly a cap structure. Additional cleavage of these intermediate products can generate a class of short RNAs, some also bearing a cap structure.

but, at the same time, contain the transcription-associated histone modification H3-triMeK4 (Bernstein et al., *Cell 125:* 315 [2006]). It has been proposed that these bivalent domains silence developmentally regulated genes in ES cells while keeping them poised for activation as cells enter the various differentiation pathways (Bernstein et al., *Cell 125:* 315 [2006]; Jorgensen et al. *Cell Cycle 5:* 1411 [2006]). Repression of H3-triMeK27 appears to be mediated by the Polycomb repression complex 2 (PRC2), which is associated with a significant number of developmental regulators.

A commonality among several of the ES-cell-specific chromatin properties is that they are all characteristic of transcriptionally active chromatin, including the reduction in heterochromatin domains, the presence of bivalent histone modifications, and the looser binding of architectural chromatin proteins. On the basis of these features, we have suggested that ES cell genomes are globally transcriptionally active and express large regions of the genome indiscriminately (Meshorer and Misteli, *Nat. Rev. Mol. Cell. Biol. 7:* 540 [2006]). In collaboration with the Misteli laboratory, we are directly testing the existence of widespread transcription in pluripotent ES cells. We provided several lines of evidence in support of global, low-level transcriptional activity over large regions of the genome. We found elevated levels of total RNA and mRNA in pluripotent mouse ES cells, and we showed that undifferentiated ES cells unexpectedly express repetitive sequences and mobile elements as well as lineage- and tissue-specific genes at low levels. Using whole-genome mouse tiling arrays, we showed that a larger fraction of the genome is active in ES cells. The global transcriptional activity of the ES cell genome was accompanied by the specific up-regulation of chromatin remodeling proteins and the global transcription machinery but not histone-modifying activities. Our results identified a novel hallmark of ES cell genomes, and they suggest that loss of pluripotency and lineage specification occurs via the reduction of the actively transcribed portion of the genome.

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STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

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E.J. Enemark C. Kuhn S. Lin
C. Faehnle P.R. Kumar T. Schalch

We study the molecular basis of nucleic acid 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 these molecular machines. Biochemistry and molecular biology allow us to study properties that can be correlated to protein structure and function.

Mechanisms of RNAi

T. Schalch, C. Faehnle, C. Kuhn, C. Kuscu, J. Calarco, S. Goldsmith, S. Lin [in collaboration with G.J. Hannon and R.A. Martienssen, CSHL; J. Partridge, St. Jude Children's Research Hospital]

RNA interference (RNAi) has made an enormous impact on biology in a very short period of time. Not only are we still discovering new cellular pathways for the regulation of gene expression, but RNAi has become an extraordinarily useful and simple tool for gene silencing. Almost from its beginnings, investigators have used genetics, biochemistry, molecular biology, and bioinformatics to study the mechanism of RNAi and related pathways. We argued, however, that in order to get a true mechanistic understanding of these pathways, we must understand how the components of the RNAi machinery worked at a molecular level. Therefore, we embarked on structural and biochemical studies of key proteins in the RNAi pathway.

During RNAi, long double-stranded RNA is processed to yield short (~19–31 nucleotide) double-stranded RNAs that trigger the RNAi response. These short RNAs get incorporated into effector complexes called the RNA-induced silencing complex (RISC), where in the mature complexes, a single-stranded RNA—the antisense strand of the original double-stranded RNA—is retained in the complex. This short RNA (short interfering RNA or microRNA) then acts to guide the RISC complex to its target through base complementarity. The best-characterized pathway, and the one that is predominantly used for gene knockdown technology, is a posttranscriptional silencing (PTGS) pathway called "slicing." Here, the RISC complex is targeted to the mRNA and produces an endonucleolytic cut in the mRNA target, thus preventing gene expression from proceeding. Other RNAi silencing pathways such as translational inhibition and transcriptional gene silencing (TGS) are also mediated through RISC complexes. In all cases, these complexes contain a small single-stranded RNA and an Argonaute protein, features that serve to define the RISC complex.

In the past few years, we have been studying Argonaute family proteins, their complexes, and their roles in various RNAi silencing pathways.

DNA Translocation in a Replicative Hexameric Helicase

E.J. Enemark

During DNA replication, two complementary DNA strands are separated and each becomes a template for the synthesis of a new complementary strand. Strand separation is mediated by a helicase enzyme, a molecular machine that uses the energy derived from ATP hydrolysis to separate DNA strands while moving along the DNA. Recently, we determined a crystal structure of the replicative helicase E1 from papillomavirus bound to single-stranded DNA and nucleotide molecules at the ATP-binding sites.

Papillomaviruses are tumor viruses that cause benign and cancerous lesions in their host. Replication of papillomavirus DNA within a host cell requires the viral E1 protein, a multifunctional protein. E1 initially participates in recognizing a specific replication origin DNA sequence as a dimer with E2, another viral protein. Subsequently, further E1 molecules are assembled at the replication origin until two hexamers are established. These hexamers are the active helicases that operate bidirectionally in the replication of the viral DNA. To unwind DNA, helicases must separate the two strands while moving along or translocating on the DNA. On the basis of structures of the DNA-binding domain of E1 bound to DNA that we determined a few years ago

in collaboration with Arne Stenlund's lab here at CSHL, we suggested a mechanism for DNA strand separation. However, the mechanism that couples the ATP cycle to DNA translocation has been unclear.

The E1 hexameric helicase adopts a ring shape with a prominent central channel. ATP-binding (and hydrolysis) sites are located at the subunit interfaces, and multiple configurations are observed within the hexamer. These have been assigned as ATP-type, ADP-type, and apo-type. The configuration of the site for a given subunit correlates with the relative height of its DNA-binding hairpin in the staircase arrangement. The subunits that adopt an ATP-type configuration place their hairpins at the top of the staircase, whereas the hairpins of apo-type subunits occupy the bottom positions of the staircase. The hairpins of the ADP-type subunits are placed at intermediate positions.

A straightforward "coordinated escort" DNA-translocation mechanism is inferred from the staircased DNA binding and its correlation with the configuration at the ATP-binding sites. Each DNA-binding hairpin maintains continuous contact with one unique nucleotide of single-stranded DNA and migrates downward via ATP hydrolysis and subsequent ADP release at the subunit interfaces. ATP hydrolysis occurs between subunits located toward the top of the staircase, whereas ADP release occurs between subunits located toward the bottom of the staircase. The hairpin at the bottom of the staircase releases its associated single-stranded DNA phosphate to conclude its voyage through the hexameric channel. Upon binding a new ATP molecule, this subunit moves to the top of the staircase to pick up the next available single-stranded DNA phosphate, initiating its escorted journey through the channel and repeating the process. For one full cycle of the hexamer, each subunit hydrolyzes one ATP molecule, releases one ADP molecule, and translocates one nucleotide of DNA through the interior channel. A full cycle therefore translocates six nucleotides with associated hydrolysis of six ATPs and release of six ADPs.

We performed a detailed comparison with other multimeric ATPase motors that highlighted the roles of individual site residues in the ATPase activity.

NADP Regulates the Yeast *GAL* Induction System

P.R. Kumar [in collaboration with S.A. Johnston, Arizona State University; R. Sternglanz, Stony Brook University]

Transcriptional regulation of galactose metabolizing genes in *Saccharomyces cerevisiae* depends on three core proteins: Gal4p, the transcriptional activator that binds to upstream activating DNA sequences (UAS_{GAL}); Gal80p, a repressor that binds to the carboxyl terminus of Gal4p and inhibits transcription; and Gal3p, a cytoplasmic transducer which upon binding galactose and ATP relieves Gal80p repression. The current model of induction relies on Gal3p sequestering Gal80p in the cytoplasm. However, the rapid induction of this system implies that there is a missing factor. To understand the molecular mechanism of the *GAL* regulatory system, we have determined the structure of *S. cerevisiae* Gal80p with the activation domain of Gal4p.

The crystal structures of Gal80p reveal a three-domain architecture with an amino-terminal domain consisting of a Rossmann fold, normally associated with binding of NAD(P) cofactors. The carboxy-terminal domain consists of a large β sheet that forms an extensive dimer interface with another monomer (Fig. 1). A large cleft is apparent between these two domains. A smaller third domain, located between the amino- and carboxy-terminal domains, consists of three small β strands and a helix that resemble a set of fingers at the entrance of the cleft. Gal80p dimers form tetramers in both crystal forms. When we soaked a crystal of $Gal80p^{S0}$:P21 (a 21-amino-acid peptide that contains the conserved region of the carboxy-terminal activation domain [AD] of Gal4p) with nicotinamide adenine dinucleotide (NAD), the structure revealed that NAD binds to both monomers of Gal80p in the cleft formed by the Rossmann fold. We were also able to observe extra density corresponding to the P21 peptide. The peptide appears to interact with the nicotinamide portion of the dinucleotide. NAD nestles between Gal80p and Gal4p, making several key interactions with Gal80p.

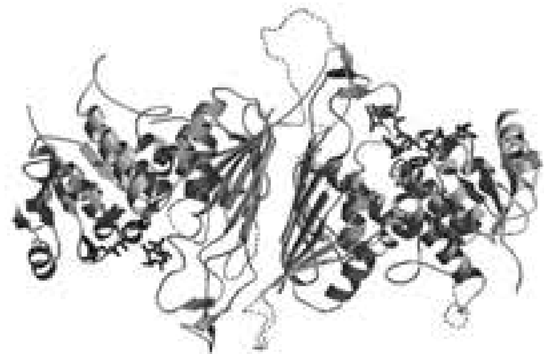

FIGURE 1 Two views of the $Gal80p^{S0}$-Gal4AD-NAD dimer. Gal80p is depicted as gray ribbons, the Gal4p AD peptide and the NAD in stick . Disordered regions are shown as a dashed coil. The β-sheet regions of the carboxy-terminal domains form an extensive dimeric interface.

In vitro pull-down assays of Gal80p with purified recombinant GST-Gal4p containing the acidic AD in the presence of NAD and NADH showed no change in binding for either of these two dinucleotides. However, when NADP and NADPH were used, a clear reduction in binding is observed with increasing concentrations of NADP. Alterations in the NAD(P)-binding site affects the initial rate of *GAL* induction in vivo, but not overall final expression levels. It appears that NAD might facilitate Gal80p binding to Gal4p, because we could only identify Gal4p-AD with NAD bound, and NADP destabilizes this interaction. The mutations, affecting both NAD and NADP binding, would therefore disrupt both the stabilizing effect of NAD and destabilizing effect of NADP with a net result of faster induction for the mutants compared to wild type.

Although we do not understand precisely how this trigger for *GAL* regulation functions, nor the involvement of NADP versus NAD, we speculate that switching the cell to a fermentable galactose medium causes a change in NADP/NADPH or NADP/NAD ratios in the cell, and Gal80p effectively senses the metabolic state of the cell. NADP might be acting as a "second messenger" in triggering the system. Alternatively, Gal80p may function as an oxidoreductase enzyme, actively converting NADPH to NADP in the presence of a substrate, causing it to disassociate from Gal4p.

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RNA SPLICING

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MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

RNA splicing is required for correct expression of most eukaryotic protein-coding genes. The spliceosome selects authentic splice sites with very high fidelity, relying on limited sequence information present throughout introns and exons. In humans, ~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 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 human genetic diseases. Related areas of interest include the remodeling of mRNP architecture as a consequence of splicing, which influences downstream events, such as nonsense-mediated mRNA decay (NMD), the role of alternative splicing misregulation in cancer, and the development of effective methods to correct defective splicing or modulate alternative splicing. A summary of some of our recently published studies is provided below.

SF2/ASF: A SPLICING FACTOR ONCOPROTEIN

Alternative splicing has an important role in cancer, partly by modulating the expression of many oncogenes and tumor suppressors, and also because inactivating mutations that affect alternative splicing of various tumor suppressor genes account for some of the inherited and sporadic susceptibility to cancer. We previously showed that the splicing factor SF2/ASF is an oncoprotein that is up-regulated in many cancers and can transform immortal rodent fibroblasts when slightly overexpressed. The mammalian target of rapamycin (mTOR) signaling pathway is activated in many cancers, and pharmacological blockers of this pathway are in clinical trials as anticancer drugs. In collaboration with Scott Lowe at CSHL, we examined the activity of the mTOR pathway in cells transformed by SF2/ASF and found that this splicing factor activates the mTORC1 branch of the pathway, as measured by S6K and eIF4EBP1 phosphorylation. This activation is specific to mTORC1, because no activation of Akt, an mTORC2 substrate, was detected. mTORC1 activation by SF2/ASF bypasses upstream phosphoinositol-3 kinase (PI3K)/Akt signaling and is essential for SF2/ASF-mediated transformation, because inhibition of mTOR by rapamycin blocked transformation by SF2/ASF in vitro and in nude mice. Moreover, short hairpin RNA (shRNA)-mediated knockdown of mTOR, or of the specific mTORC1 and mTORC2 components Raptor and Rictor, abolished the tumorigenic potential of cells overexpressing SF2/ASF. These results suggest that clinical tumors with SF2/ASF up-regulation could be especially sensitive to mTOR inhibitors.

ANTISENSE-CORRECTION OF SPLICING DEFECT IN SMA

Spinal muscular atrophy (SMA) is a neurodegenerative genetic disorder caused by the deletion or mutation of the survival-of-motor-neuron gene *SMN1*. An *SMN1* paralog, *SMN2*, is present in all patients and differs from *SMN1* by a C to T transition in exon 7 that causes substantial skipping of this exon, such that *SMN2* expresses only low levels of functional protein. We continue to explore strategies to increase the extent of exon-7 inclusion during splicing of *SMN2* transcripts, for eventual therapeutic use in SMA. One of our strategies involves the use of antisense oligonucleotides (ASOs), in collaboration with Frank Bennett (Isis Pharmaceuticals).

We systematically tested overlapping ASOs complementary to the proximal flanking intronic regions, covering 60 nucleotides upstream or downstream from exon 7. As we did previously with exonic ASOs, we classified them into positive, negative, and neutral with respect to their effects on exon-7 inclusion. Several of the positive ASOs were very potent when tested in cell-free splicing or in transfected cells, defining silencer regions in both flanking introns. We also tested them in transgenic mice that ubiquitously express human *SMN2*. After intravenous administration, 2′-O-methoxy-ethyl (MOE) ASOs are efficiently internalized by cells in peripheral tissues, and they are imported into the nucleus, where they can modulate gene expression. We observed efficient correction of the splicing defect in liver and kidney mRNAs and weaker but significant correction in skeletal muscle. There was no correction in spinal cord—the tissue we need to target for SMA therapy—because the ASOs do not cross the blood-brain barrier. Therefore, we have started to explore methods for direct administration to the central nervous system (CNS), such as intracerebroventricular infusion.

The above experiments show that ASO tiling is a powerful method to map *cis*-acting elements that influence alternative splicing. To explore the mechanism of action of the silencer element in intron 7—which corresponds to an element previously dubbed ISSN1—we introduced point mutations and assayed splicing of the resulting minigenes in transfected cells. Combined with splicing-factor overexpression and biochemical experiments, this analysis demonstrated that the intron-7 splicing silencer is composed of a bipartite motif recognized by the splicing repressor heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and other closely related members of the hnRNP A/B family.

CHARACTERIZATION OF SPLICING-REGULATORY NETWORKS

The precise regulation of many alternative splicing events by specific splicing factors is essential to determine tissue types and developmental stages. However, the molecular basis of tissue-specific alternative splicing regulation and the properties of splicing regulatory networks (SRNs) are poorly understood. In collaboration with Michael Zhang at CSHL and John Castle at Rosetta Inpharmatics, we comprehensively predicted the targets of the brain- and muscle-specific splicing factor Fox-1 (A2BP1) and its paralog Fox-2 (RBM9) and systematically defined the corresponding SRNs genome-wide. It was known that Fox-1/2 are conserved from worms to humans and specifically recognize the RNA element UGCAUG. We integrated Fox-1/2-binding specificity with phylogenetic conservation, splicing microarray data, and additional computational and experimental characterization. We predicted thousands of Fox-1/2 targets with conserved binding sites, at a false discovery rate of ~24%, including many validated experimentally, suggesting a surprisingly extensive SRN. The preferred position of the binding sites differs according to the alternative splicing pattern and determines either activation or repression of exon recognition by Fox-1/2. Many predicted targets are important for neuromuscular functions and have been implicated in several genetic diseases. We also identified instances of binding-site creation or loss in different vertebrate lineages and human populations, which likely reflect fine-tuning of gene expression regulation during evolution.

SPLICE-SITE RECOGNITION AND HUMAN GENETICS

We continued to analyze the specificity of splice-site recognition and the relationship between splice-site mutations and genetic diseases. The 5′ splice site—the highly diverse element at the 5′ end of introns—is initially recognized via base pairing to the 5′ end of the U1 small nuclear RNA (snRNA). However, many natural 5′ splice sites have a poor match to the consensus sequence and are predicted to be weak. Using genetic suppression experiments in human cells, we demonstrated that some atypical 5′ splice sites are actually efficiently recognized by U1, in an alternative base-pairing register that is shifted by one nucleotide relative to the canonical one (Fig. 1). These atypical 5′ splice sites are phylogenetically widespread, and many of them are conserved. The unexpected flexibility in 5′ splice site/U1 base pairing challenges an established paradigm and has broad implications for splice-site prediction algorithms and gene-annotation efforts in genome projects.

In addition, shifted base pairing provides an explanation for the effect of a 5′ splice-site mutation in intron 2 of *RARS2*, which is associated with the genetic disorder pontocerebellar hypoplasia. This mutation was known to cause defective splicing, which was paradoxical because the mutation changes a nonconsensus nucleotide to a consensus nucleotide. However, because this atypical 5′ splice site base pairs with U1 in a shifted register, the mutation weakens this interaction, accounting for the splicing defect.

In many other cases, including particular alleles of Fanconi anemia, hemophilia B, neurofibromatosis, and phenylketonuria, mutations can disrupt splicing even though they do not disrupt the match to the splice-site consensus. In collaboration with Ravi Sachidanandam at CSHL, we used comparative genomics—i.e., analysis of large-scale data sets of splice sites from five different

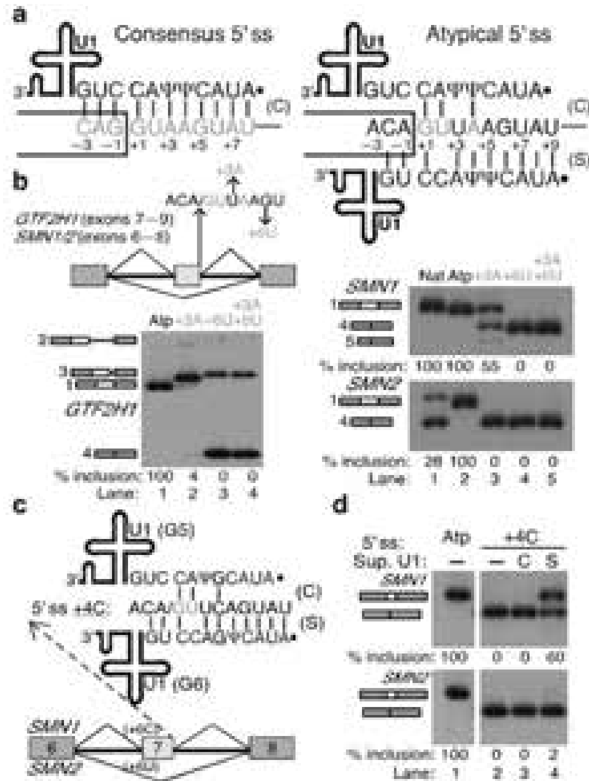

FIGURE 1 Shifted base pairing between atypical 5′ splice sites and the 5′ end of U1 small nuclear RNA. (*a*) Diagram of two base-pairing registers between the 5′ splice sites (positions are numbered) and U1. (*Light gray*) Consensus nucleotides; (ψ) pseudouridine; (*closed dot*) 2,2,7-trimethylguanosine cap at the 5′ end of U1; (*boxed area*) upstream exon; (*solid line*) intron. (*Vertical lines*) Base pairs in the canonical (C) or shifted (S) register. Note that the atypical 5′ splice site can form seven more base pairs to U1 in the shifted arrangement. (*b*) Mutations at the atypical (Atp) 5′ splice site that disrupt shifted but enhance canonical base pairing abolish correct splicing. The human *GTF2H1* and *SMN1/2* minigenes are schematically represented at the top, indicating the mutations introduced at the atypical 5′ splice site. *GTF2H1* intron 8 has a natural atypical 5′ splice site; intron 7 in *SMN1* or *SMN2* has a canonical 5′ splice site, but we replaced it in both genes to allow testing of the atypical 5′ splice site in two heterologous contexts. The identity of the various spliced mRNAs, detected by radioactive reverse transcriptase–polymerase chain reaction (RT-PCR), is schematically shown on the left of the gels: (1) correctly spliced mRNA; (2) retention of the downstream intron; (3) use of a cryptic 5′ splice site in the middle exon; (4) skipping of the middle exon; (5) activation of a cryptic 5′ splice site in the first exon. (*Bottom*) Percentage of correct splicing. (Nat) Natural *SMN1/2* exon-7 5′ splice site. (*c*) Compensatory U1 mutations that restore shifted but not canonical base pairing rescue splicing at atypical 5′ splice sites. *SMN1/2* minigenes carrying the +4C mutation at a heterologous atypical 5′ splice site in exon 7 were cotransfected with suppressor U1 small nuclear RNAs. The 5′ end of U1 was mutated so as to rescue base pairing in the canonical or the shifted arrangement. Mutations are underlined. (*d*) RT-PCR analysis of RNAs from the suppressor U1 experiment. Labels on top indicate the suppressor U1 in either register. (*Bottom*) Percentage of exon-7 inclusion.

genomes—to identify pairwise dependencies between individual nucleotides of the 5′ splice site as a conserved feature of the entire set of 5′ splice sites. These dependencies are also conserved in human– mouse pairs of orthologous 5′ splice sites. Many disease-associated mutations disrupt these dependencies, as can some human single-nucleotide polymorphisms (SNPs) that appear to alter splicing, suggesting that 5′-splice-site SNPs have a role in complex diseases.

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THE DYNAMIC TUMOR MICROENVIRONMENT

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Bone marrow (BM)-derived components of the tumor microenvironment serve critical roles in regulating tumor growth and metastasis. Tumor-derived paracrine signals instigate the BM compartment that mobilize and recruit discrete subsets of BM cells to the tumor bed. Recruited proangiogenic BM-derived cells contribute significantly to neovasculature formation and tumor growth in adults. In addition to the perivascular contribution of BM-derived hematopoietic cells, the BM-derived endothelial progenitor cells (EPCs) provide an additional alternative source of endothelial cells that contribute to neovessel formation. In response to tumor cytokines, including vascular endothelial growth factor (VEGF), putative VEGF receptor-2 (VEGFR2)-positive EPCs mobilize into the peripheral blood circulation, subsequently move to the tumor bed, and incorporate into sprouting neovessels.

More recent investigations have shown that EPCs participate in neovascularization during acute ischemic injury in both humans and mice. For example, Minami et al. (*Circulation 112:* 2951 [2005]) have shown that circulating endothelial cells engraft luminally into 15%–29% of the vessels of the transplanted human heart. BM-derived endothelial cells have also been shown to give rise to up to 16% of the neovasculature in spontaneous tumors growing in transgenic mice and also contribute to human tumor vessels. However, since the first description of EPCs, their identity and relative contribution to neovasculature formation have often been debated. Much confusion has prevailed because of the extensive variability in EPC contribution to vessel formation in a variety of tumor model systems. The recent controversy notwithstanding, the existence of a BM reservoir of EPCs and their selective involvement in neovascularization have attracted considerable interest because these cells not only represent a novel target for therapeutic intervention but are also being used successfully as surrogate markers for monitoring cancer progression, as well as for optimizing efficacy of antiangiogenic therapies such as anti-VEGFR2 antibody therapy.

The BM compartment comprises the osteoblastic (or endosteal) and vascular niches. The osteoblastic niche provides a quiescent microenvironment for stem cell maintenance, and the resident hematopoietic stem cells (HSCs) are anchored to the endosteal surface by calcium-sensing receptors present on the HSC. Growing tumors secrete soluble factors into the circulation that switches the marrow microenvironment from a quiescent state to a highly proangiogenic and protumorigenic environment. This in turn promotes the mobilization of both vascular and hematopoietic progenitors to the peripheral circulation, which are recruited to the tumors. In the tumor bed, the BM-recruited cells and other stromal cells (adipocytes, fibroblasts, etc.) constitute a unique microenvironment that can modify the neoplastic properties of the malignant tumor cells.

Adult BM contributes significantly to endothelial and lymphatic neovessel formation and tumor growth and invasion. Among the BM-derived cells, much focus has been directed toward the proangiogenic hematopoietic mural cells that are recruited to the tumor bed, where they exert their functions perivascularly via paracrine release of proangiogenic cytokines. Several populations of BM-derived hematopoietic cells have been reported to contribute to tumor angiogenesis and invasion. These include $GR1^{+}CD11b^{+}$ myeloid progenitors, tumor-associated macrophages (TAMs), Tie2-expressing monocytes (TEMs), $CXCR4^{+}VEGFR1^{+}$ hemangiocytes, recruited BM-derived circulating cells, $PDGFR^{+}$ pericyte progenitors, vascular leukocytes, and infiltrating mast cells and neutrophils. Despite the general importance of these cells in tumor angiogenesis, the precise contribution and biological function of specific lineages remain poorly understood.

In addition to the perivascular contribution of BM-derived hematopoietic cells, the BM-derived EPCs provide an additional source of endothelial cells that contribute to neovessel formation. We have characterized EPCs with a comprehensive set of endothelial, hematopoietic, and progenitor markers (Nolan et al. *Genes Dev. 21:* 1546 [2007]). Analysis of EPCs in the BM, peripheral blood, and tumors grown in mice that were previously transplanted with green fluorescent protein $(GFP)^{+}$ BM required the use of a distinct combination of cell surface markers. In early tumors, BM-derived EPCs were identified as GFP^{+} cells expressing VE-cadherin (uniformly on the cell surface), $CD31^{dim}$, and Prominin I/AC133. These cells also expressed VEGFR2 and lacked hematopoietic

markers including CD11b, CD45B220, and CD41. Notably, whether EPCs are $CD45^{-}$ or $CD45^{dim}$ remains to be clarified. In this context, it may be important to determine the status of CD45 on EPCs in the context of multiple CD45 isoforms, CD45RA, CD45RB, CD45RO, and CD45B220. Regardless, the ability of isolated EPCs to differentiate into mature endothelial cells and luminally incorporate into sprouting neovessels in vitro was used as a functional readout. Isolectin ensured luminal incorporation, GFP validated BM derivation, CD31 confirmed endothelial cells, and CD11b gated out any hematopoietic contamination in the CD31 channel. This was critical because CD31 is expressed by a subset of hematopoietic cells. Similarly, circulating EPCs in the peripheral blood were detected as GFP^{+} c-Kit^{+} $VEGFR2^{+}$ VE-$cadherin^{+}$ $CD11b^{-}$ cells. In this scenario, c-*kit* (progenitor marker) distinguished BM-derived EPCs from circulating endothelial cells that have sloughed off from mature vessels.

Indeed, luminally incorporated BM-derived endothelial cells determined by high-resolution microscopic analysis of multiple Z-stacks (resolution of at least 0.275–0.35 μm, depth of 30 μm) and optical sectioning have shown that the BM-derived endothelial cells have a single nucleus and that the GFP and CD31 signals are localized to the same individual cell, indicating that the incorporated endothelial cell is derived from the BM. Notably, the vessel-incorporated BM-derived endothelial cells exhibit hallmarks of a typical mature endothelial cell such as uniform surface expression of CD31, a characteristic VE-cadherin staining at the intercellular adherens junctions, intracellular GFP, and lack of hematopoietic markers.

A systematic analysis of EPC contribution to implanted tumors (Lewis lung carcinoma, B6RV2 lymphoma, melanoma), spontaneous breast tumors arising in mouse mammary tumor virus-polyoma middle T (MMTV-PyMT) mice, and pulmonary metastases that develop in these mice has shown that ~10%–20% of the early tumor vessels were BM derived. So how does one explain the extreme variability in EPC contribution to tumor vessels in various published studies? A possible explanation is that in addition to tumor-type dependence, it is possible that the EPC contribution could be tumor-stage specific. Indeed, a kinetic analysis of EPC contribution as a function of tumor growth showed that EPCs are recruited to early tumors preceding vessel formation, followed by differentiation into endothelial cells and luminal incorporation into a subset of sprouting tumor neovessels. Noticeably, in growing tumors, these chimeric BM-derived vessels were eventually diluted/replaced with non-BM-derived host vessels, thereby explaining the low contribution observed in large established tumors in various studies.

To directly address their functional role in supporting tumor angiogenesis in vivo, acute and conditional shRNA-mediated silencing of Id1 mRNA in the adult BM resulted in EPC mobilization defects associated with severe angiogenesis inhibition and impaired growth of primary tumors and progression of micrometastasis to macrometastasis, suggesting a critical role for these cells in angiogenesis-mediated tumor growth. Alternatively, targeting transiently expressed monomeric VE-cadherin specifically on EPCs using radiolabeled anti-VE-cadherin antibody resulted in severe angiogenesis inhibition and impaired tumor growth. So, why is this relatively minor contribution so critical for tumor growth? Notably, in addition to vessel incorporation, we have shown that tumor-recruited EPCs secrete proangiogenic growth factors, suggesting that in addition to providing stability to nascent vessels, EPCs have a paracrine role in vessel recruitment at a critical early stage of tumor growth.

EPCs contribute significantly to tumor neoangiogenesis, and their selective targeting may have broad implications for the development of antiangiogenic cancer therapy. EPCs are being considered as useful surrogate markers for monitoring cancer progression, as well as for optimizing the efficacy of antiangiogenic therapies, such as anti-VEGFR2 antibody therapy. Recently, because of interest in the role of EPCs in turning on the "angiogenic switch," strategies have been used in an attempt to keep this switch in the "off" position.

Our finding that EPCs are the main regulators of the angiogenic switch in metastatic progression suggests that their selective targeting may be a promising approach in cancer patients where metastatic colonization to the lung has already occurred; this represents a paradigm shift and a foundation for very exciting clinical applications. Given that in many cancer patients, metastatic spread has already occurred by the time of primary diagnosis, this study suggests that selectively targeting the angiogenic switch via the endothelial progenitor cells may provide a clinically feasible approach to blocking metastasis progression and preventing death in cancer patients. For example, following resection of primary tumors, patients with stage-3 breast or colon cancers are often treated with chemotherapy to destroy dormant micrometastases. Nonetheless, many of these patients frequently succumb to progression of micrometastatic invasive tumors. Thus, combinations of adjuvant chemotherapy with antiangiogenic agents to block recruitment of the endothelial progenitor cells provide a highly effective strategy to impair establishment of metastatic lesions.

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CELL BIOLOGY OF THE NUCLEUS

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Most cellular processes can trace their beginnings to the nucleus where a gene is activated, resulting in the production of an RNA molecule that must get processed and transported to the cytoplasm. Although much biochemical information is available regarding many of the factors involved in gene expression, the spatial and temporal aspects of gene expression and the dynamics of the nuclear domains that the gene expression machinery occupies are less well understood. During the past year, we have focused on two main areas: (1) examining the dynamics of gene expression/repression and DNA repair in living cells and (2) characterizing the role of noncoding RNAs in regulating gene expression.

The Dynamics of Gene Expression/Repression and DNA Repair

M. Hübner, R. Ileng Kumaran, J. Li, R. Zhao

Our research during the past year has focused on the spatial and temporal aspects of gene expression in living cells. These studies were performed using a cell line (U2OS 2-6-3) that contains a transgene stably integrated as a 200-copy array in human chromosome 1p36 (Janicki et al., *Cell 116:* 683 [2004]). This cell system allows us to directly visualize gene expression (DNA, RNA, protein) within the context of a living cell. During the past year, using 4D–time lapse microscopy, we have compared the kinetics at which the transcription machinery is recruited to a specific genetic locus upon transcriptional initiation during interphase to that observed when transcription is initiated immediately after mitosis. In interphase, after doxycycline addition (1 mg/ml) to induce the locus, we observed decondensation of the locus from a heterochromatic state to a euchromatic state, followed by the accumulation of RNA polymerase II (RNA pol II) at the locus and the production of nascent transcripts. By plotting the relative fluorescence signal intensity of the locus over time, we found that RNA pol II levels and mRNA production gradually increased, taking ~180 min to reach a maximal signal intensity. We similarly studied the kinetics of transcriptional initiation of this genetic locus upon exit from mitosis. In this case, we keep the locus in a transcriptionally active state and allow it to naturally shut down upon entry into mitosis. Upon exit from mitosis, in the presence of doxycycline, RNA pol II was recruited to the locus after nuclear envelope reformation in telophase, and mRNA synthesis was first detected ~3–5 min later. In contrast to the relatively slow, gradual kinetics of induction observed in interphase, RNA pol II reached its maximal signal intensity at the locus in only 2–4 min after transcriptional activation, with mRNA production peaking at 15–20 min. Given that the transcript length of the RNA derived from this locus is 3.4 kb and that transcription occurs at ~1.5 kb/min, we are able to detect a relatively low level of transcripts at the locus upon induction. On the basis of these data, we conclude that the transcriptional machinery is recruited to this same genetic locus with very different kinetics depending on the state of the cell cycle. In particular, we observed that a heterochromatic locus requires markedly more time to be activated versus a locus that is constitutively induced. Studies are currently under way to examine the histone modifications that are present at this locus during interphase and mitosis in order to determine if particular histone modifications are involved in mediating the observed difference in transcriptional induction. Our study provides a real-time view of interphase transcriptional induction and post-mitotic reactivation at a single gene locus and provides a system in which to study how gene expression patterns are transmitted to daughter cells.

A second focus of our studies has been to examine the assembly of the NHEJ (nonhomologous end-joining) DNA-repair nanomachine in living cells at high resolution in order to determine the mechanism by which this nanomachine assembles at the site of DNA breaks, its residence time at the break, and to elucidate the process by which it disassembles upon completion of the repair process in interphase nuclei. An in-depth understanding of the assembly and dynamics of the NHEJ DNA-repair nanomachine will have significant relevance to understanding and regulating this process. In collaboration

with Matthew Porteus (University of Texas Southwestern Medical School), we have used engineered chimeric zinc finger nucleases (ZFNs) that combine the nonspecific cleavage domain of the Fok I restriction enodnuclease with custom-designed zinc finger domains, specific to unique DNA sequences. Using this approach, we first examined whether the cyan fluorescent protein (CFP) gene encoded in our transgene array could be cleaved by the ZFNs to induce double-stranded breaks (DSBs), resulting in the recruitment of the DSB-repair nanomachine to the reporter locus. To do so, we first transiently transfected U2OS-2-6-3 cells with LacI-YFP (yellow fluorescent protein) to visualize the locus and ZFN1 and ZFN2 expression plasmids for 24 h. Only when both ZFN subunits are expressed is a functionally active complex formed. Deconvolution microscopy showed that when both subunits of the ZFN were expressed, they colocalized with the stably integrated locus containing the CFP gene. Next, we were interested in determining if the localization of the ZFNs at this specific site in the human genome resulted in DSBs as indicated by the recruitment of the NHEJ/HR repair machinery. We expressed mCherry-53BP1, a well-validated upstream factor that is recruited in multiple copies to the sites of DSBs before repair is initiated, in cells that expressed either one or both subunits of the wild-type ZFN. Importantly, we could observe accumulation of mCherry-53BP1 at the LacI-EYFP-marked locus only when both ZFN1 and ZFN2 subunits were expressed simultaneously, and not in the control cells that did not express ZFNs or that expressed only ZFN1 or ZFN2 independently. In a similar experiment, we observed the localization of endogenous phospho (Ser-32)-H2A.X, another marker protein for DSB repair, at the locus by indirect-immunofluorescence analysis, when LacI-mCherry, ZFN1, and ZFN2 were expressed for 24 h in U20S-2-6-3 cells. In summary, these experiments demonstrate that ZFN technology can be applied to visualize DSB repair at a single location in the genome/nucleus of mammalian cells. Ongoing studies are focused on examining the recruitment of other DNA-repair proteins, including Ku80 and XRCC4, and visualizing the dynamics of DNA damage and repair at the site of a single copy gene.

In addition to examining the dynamics of the gene expression and DNA-repair machineries, we have also been interested in the silencing of endogenous genes by Polycomb (PcG) proteins. PcG group proteins can be divided into members of the initiation complex PRC2 (Polycomb repressive complex 2), which is involved in establishing gene silencing, and members of the maintenance complex PRC1, which maintain gene silencing during cell division. Some members of the PRC1 complex are present in nuclear structures called PcG bodies. These bodies are associated with regions of facultative heterochromatin and colocalize with chromatin enriched in histone H3 dimethylated and trimethylated at lysine 27 (H3K27). Because of the role of H3K27 methylation in gene silencing, PcG bodies have been implicated in the mitotically stable inheritance of the inactive state of genes. PcG bodies can be found in a wide range of cell types from cancer cell lines to primary human and mouse cell types and tissue samples from cancer patients. They are present throughout the cell cycle, and they attach to chromosomes during mitosis, thereby providing a possible mechanism for epigenetic gene silencing. PcG bodies are enriched in the phosphorylated form of the PRC2 protein Bmi1. During the past year, we identified a single site (S258) responsible for the majority of Bmi1 phosphorylation. Interestingly, mutation of single or multiple putative phosphorylation sites did not prevent Bmi1 from localizing to PcG bodies. However, when stably expressed in mouse embryonic stem cells devoid of the Bmi1 protein, the Bmi1 mutants lead to a misregulation of Hox genes that are crucial for cellular differentiation and embryonic development. This suggests that Bmi1 phosphorylation has a role in the regulation of Hox gene expression. We have previously shown that tethering H3K27 methyltransferase EZH2 to a genetic locus leads to H3K27 trimethylation, gene silencing, and the formation of a PcG body. We are currently expanding these studies to regulate an endogenous gene. In conclusion, we have developed several in vivo approaches to examine the dynamics of gene expression/silencing and DNA repair in living cells. Ongoing studies will elucidate the underlying principles of these dynamics and how they can be manipulated in vivo to regulate gene expression.

Identification and Characterization of Nuclear Retained Noncoding RNAs

H. Sunwoo, J. Wilusz

Although it has been generally assumed that most genetic information is expressed as and transacted by proteins, recent evidence from genomic tiling arrays and large-scale cDNA-cloning projects suggests that the majority of transcriptional output of the mammalian genome represents RNA that does not code for proteins. These noncoding RNAs (ncRNAs) include microRNAs, Piwi-interacting RNAs (piRNAs), and small nucleolar RNAs (snoRNAs) as well as a significant number of longer transcripts, most of whose functions are

unknown. These longer transcripts probably do not simply represent transcriptional "noise" because many have been shown to exhibit cell-type-specific expression, localization to specific subcellular compartments, and association with human diseases. Therefore, the big and largely unanswered question that we are trying to address is, What are the functions of these long noncoding RNAs?

Using microarray analysis in collaboration with J. Mattick's laboratory (Institute for Molecular Bioscience, University of Queensland, St Lucia, Australia), we have examined ncRNAs whose expression levels change after mouse C2C12 myoblasts differentiate into myotubes. RNA fluorescence in situ hybridization (FISH) analysis of candidate clones revealed one clone, corresponding to the MEN ε/β locus, which is up-regulated 3.3-fold upon myoblast differentiation to myotubes. Two ncRNA isoforms are produced from a single RNA pol II promoter, differing in the location of their 3′ ends. MEN ε is a 3.2-kb polyadenylated RNA, whereas MEN β is a ~20-kb transcript containing a genomically encoded poly(A)-rich tract at its 3′ end. These RNA transcripts are broadly expressed in adult mouse tissues and conserved among mammals. The 3′ end of MEN β is generated by RNase P cleavage similar to what we have observed for MALAT1 (see below). The MEN ε/β transcripts are localized to nuclear paraspeckles and directly interact with the 54-kDa nuclear RNA-binding protein (p54/nrb). Knockdown of MEN ε/β expression results in the disruption of nuclear paraspeckles. Furthermore, the formation of paraspeckles, after release from transcriptional inhibition by DRB treatment, was suppressed in MEN ε/β-depleted cells. Our findings indicate that the MEN ε/β ncRNAs are essential structural/organizational components of paraspeckles. Ongoing studies are examining the mechanism by which these ncRNAs are retained in the nucleus and their potential function in myoblast differentiation.

MALAT1 is a long ncRNA that was originally identified as a transcript whose expression predicted metastasis of non-small-cell lung tumors and was subsequently shown to be overexpressed in many human cancers (for review, see Prasanth and Spector, *Genes Dev. 21:* 11 [2007]). This ncRNA came to our attention because it specifically localizes to nuclear speckles. Previous studies from our laboratory had indicated that a population of poly(A)$^+$ RNAs was present in nuclear speckles, but the precise RNAs have remained unknown. During the past year, we became interested in whether small RNAs may be generated from the MALAT1 locus. Our pursuit of this question resulted in the identification of a previously unrecognized 3′-end processing mechanism by which a single nascent transcript can yield two ncRNAs with distinct subcellular localizations. We found that the previously reported ~7-kb MALAT1 transcript is actually cleaved to produce a nuclear-retained ~6.7-nucleotide transcript that is localized to nuclear speckles and a 61-nucleotide RNA that is found exclusively in the cytoplasm (and thus we named it mascRNA, *M*ALAT1-*a*ssociated *s*mall *c*ytoplasmic RNA). We have shown that the ~6.7-kb nuclear-retained ncRNA is involved in regulating the level of pre-mRNA splicing factors that are recruited to an inducible genetic locus. Ongoing studies are examining the mechanism by which this regulation is occurring. Unexpectedly, the 61-nucleotide mascRNA was found to fold into a cloverleaf structure that is recognized by RNase P which cleaves its 5′ end, simultaneously generating the 3′ end of the mature MALAT1 transcript and the 5′ end of mascRNA. Upon cleavage of the 3′ end of mascRNA by RNase Z, CCA is added posttranscriptionally to its 3′ end. The folding of mascRNA and the mechanism by which it is cleaved indicate that it is a tRNA-like small RNA. Although predicted to fold similarly to a tRNA cloverleaf secondary structure, mascRNA has a poorly conserved anticodon loop and it is not aminoacylated in vivo, suggesting that it does not function as a classic tRNA. Additionally, unlike tRNAs that are transcribed by RNA pol III, mascRNA is generated via processing of MALAT1, an RNA pol II transcript. Although the 3′ end of MALAT1 is not generated by a classical cleavage/polyadenylation mechanism, the mature MALAT1 transcript does have a poly(A)-tail-like moiety at its 3′ end. This results from a conserved short poly(A)-rich motif that is encoded in the genome immediately upstream of the RNase P cleavage site. These findings have revealed a new paradigm for how the 3′ ends of certain RNA pol II transcripts are produced. In addition, they suggest a general mechanism by which genetic loci are able to generate multiple ncRNA transcripts, each of which localizes to different subcellular compartments. Together, these studies have provided new insights into the generation and function of ncRNAs.

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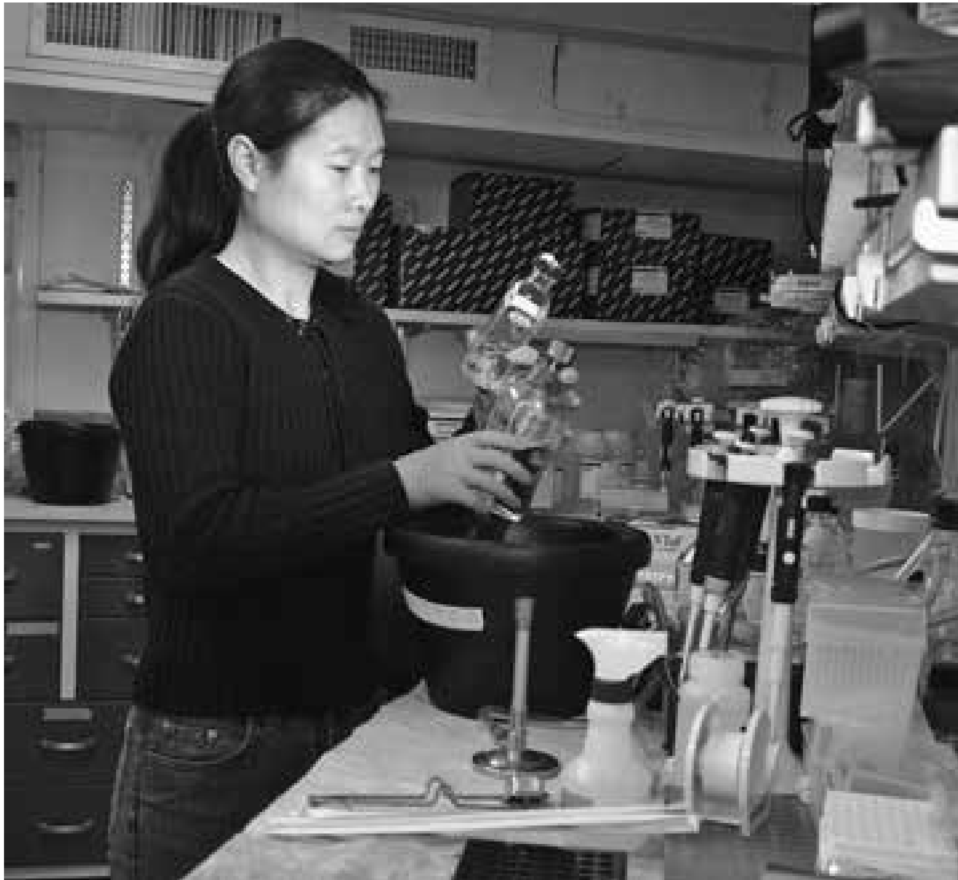

Jingjing Li

MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

A. Stenlund X. Liu
S. Schuck

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

An impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for analysis of the viral life cycle. These viruses normally require specialized differentiating cells that only with difficulty can be generated in cell culture. However, for a bovine papillomavirus (BPV-1), a convenient cell culture system exists in which 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 papillomaviruses show some unique and interesting characteristics. As part of their normal life cycle, these viruses can exist in a state of latency that is characterized by maintenance of the viral DNA as a multicopy plasmid in infected cells. The copy number of the viral DNA is tightly controlled, and the viral DNA is stably inherited under these conditions. Papillomaviruses therefore provide a unique opportunity for studying 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 of the characteristics of an initiator protein, including ori recognition, DNA-dependent ATPase activity, and DNA helicase activity. The transcription factor E2, whose precise function has remained more elusive, appears to serve largely as a loading factor for E1. Through direct physical interactions with both E1 and the ori, E2 provides sequence specificity for the formation of the initiation complex.

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

ATP BINDING AND HYDROLYSIS BY THE E1 INITIATOR PROTEIN

The E1 protein is a member of the AAA^+ family of proteins and can bind and hydrolyze ATP. The ATP-binding pocket is formed between two adjacent subunits in E1 oligomers. Analysis of the structure of the E1 helicase and oligomerization domain has revealed that a total of ten residues are likely to be involved in ATP binding and hydrolysis. To determine the precise function of these residues in ATP binding and hydrolysis, we mutated them to alanine, purified the mutant proteins, and analyzed them for the ability to bind and hydrolyze ATP. All mutant proteins were structurally intact based on their ability to form the $E1_2E2_2$–ori complex, whose formation does not require ATP. As expected, all of the mutants, with one exception (Y534A), showed severe defects for ATPase activity, demonstrating a defect in either binding or hydrolysis of ATP. To distinguish between the ability to bind and to hydrolyze ATP, we tested the mutants for E1 trimer and double trimer (DT) formation, which requires ATP binding, but not hydrolysis. Six of the mutants—K425A, K439A, S440A, D497A, Y499A, and R538A—were defective for trimer formation, demonstrating that these mutants are defective for ATP binding. The remaining four

mutants—D478A, D479A, N523A, and Y534A—were active for trimer and DT formation, demonstrating that these mutants can bind ATP. Interestingly, two of these mutants, D478A and N523A, showed significant DT formation even in the absence of nucleotide, indicating that these mutations generate a conformational change that mimics the nucleotide-bound state. These results are important because they demonstrate that the residues required for ATP binding and hydrolysis in the hexamer structure are identical to the residues that we find are required for ATP binding and hydrolysis in the DT, clearly demonstrating that the same mode of ATP binding is used in both types of complexes.

INTERACTION BETWEEN THE E1 AND THE E2 PROTEINS IN THE $E1_2E2_2$–ORI COMPLEX

An interaction between the E1 and E2 proteins on the origin of DNA replication is essential for initiation of replication by papillomaviruses. This interaction provides sequence specificity for E1 by inhibiting the nonspecific DNA-binding activity present in the E1 helicase domain. Although we have an excellent understanding of the nature of the $E1_2E2_2$–ori complex from existing DNA cocrystal structures of both the E1 DNA-binding domain (DBD) and the E2 DBD, as well as a structure of the E1 helicase domain in complex with the E2 *trans*-activation domain, we currently have a very limited understanding of how this complex, which lacks replication-related activities, is subsequently converted into the larger E1 complexes, such as the E1 DT, which can open up the DNA duplex. We have previously been able to show that although ATP is not required for the formation of the $E1_2E2_2$–ori complex, ATP is required for the transition from the $E1_2E2_2$–ori complex to larger complexes that contain only E1. To analyze the effect of ATP on the $E1_2E2_2$–ori complex, we performed experiments by measuring the half-life of the $E1_2E2_2$–ori complex in the absence and presence of nucleotide. Unexpectedly, the half-life of the $E1_2E2_2$–ori complex is very short in the absence of nucleotide (~3 min) and is extended significantly in the presence of ATP (~16 min). ATP-γ-S, which is hydrolyzed very slowly by E1, has an effect similar to that of ATP, indicating that the longer half-life of the $E1_2E2_2$–ori complex depends on nucleotide binding, but not hydrolysis. These results show that the $E1_2E2_2$–ori complex is capable of binding ATP but fails to hydrolyze ATP, likely because the two E1 molecules in the $E1_2E2_2$–ori complex are not oriented in a way that allows formation of the E1-E1 interface responsible for hydrolysis of ATP.

To test this hypothesis, we used the complete set of mutants that we generated in the E1 ATP-binding pocket. These ten residues are involved in nucleotide contacts for ATP binding and hydrolysis. The mutants fall into two groups: mutants in the A side that include the Walker A and B elements and mutants in the B side that include, for example, the arginine finger. We tested mutants on the A side for effects on the half-life of the $E1_2E2_2$–ori complex and found that the mutants K439A and S440A showed only a slight increase in half-life in the presence of ATP, consistent with a defect in binding ATP of these mutants. In contrast, mutations on the B side (exemplified by R538A) showed the same ATP-dependent increase in half-life observed for wild-type E1, indicating that the B side is not involved in binding ATP in the $E1_2E2_2$–ori complex. These results indicate that ATP is bound exclusively to the A side in the $E1_2E2_2$–ori complex, presumably because the orientation of the two helicase domains in complex with E2 does not allow the A and B sides of E1 to interact. Another mutant, D478A in the Walker B motif, has an altered conformation that mimics the ATP-bound state even in the absence of nucleotide. We tested this mutant for $E1_2E2_2$–ori complex formation in the absence and presence of nucleotide. Interestingly, D478A displayed a long half-life even in the absence of nucleotide, consistent with the idea that it is the conformation of E1 that controls the half-life of the $E1_2E2_2$–ori complex.

These data allow us to propose a mechanism for the release of E2 from the $E1_2E2_2$–ori complex. When E1 and E2 are bound together in the presence of ATP, the complex has a long half-life. If ATP is hydrolyzed and released, the resulting short half-life would allow displacement of E2. However, in order for the bound ATP to be hydrolyzed, additional E1 molecules must be added to generate an A–B interface that can hydrolyze the bound ATP. We have previously demonstrated that the ori contains four E1-binding sites (E1 BS) and that only two of these, E1 BS 2 and 4, are occupied in the $E1_2E2_2$–ori complex. Recruitment of additional E1 molecules is therefore likely to occur through binding of E1 to the E1 BS 1 and 3, which would allow the formation of an A–B interface that is competent for ATP hydrolysis. Thus, the recruitment of additional E1 molecules would serve as the trigger for the simultaneous displacement of E2 and the assembly of larger E1 complexes, such as the E1 DT.

To understand the mechanism by which ATP hydrolysis might destabilize the $E1_2E2_2$–ori complex, we combined structural information from two different sources. The BPV E1 helicase domain structure in the absence and presence of nucleotide is present in the E1 hexamer struc-

ture. We aligned the BPV E1 structures with and without nucleotide to determine which regions of the helicase domain change in the presence of ATP. Two elements change their conformation dramatically when the structures with and without ATP are compared. The first, the β-hairpin, is involved in sequence-non-specific DNA binding by the E1 helicase domain as we have recently demonstrated, and the second element, loop 2, has unknown function but shows protein contacts with the E2 activation domain in the E1E2 cocrystal structure. Interestingly, loop 2 is in close proximity to the carboxy-terminal part of the E2 activation domain, and in the ATP bound state, loop 2 is oriented toward the E2 *trans*-activation domain, whereas in the absence of nucleotide, loop 2 is twisted away from the E2 *trans*-activation domain. Loop 2 therefore represents a prime candidate for a trigger that alters the half-life of the $E1_2E2_2$–ori complex in response to ATP binding by E1.

DNA REPLICATION AND CHROMATIN INHERITANCE

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Research continues to focus on the mechanism of initiation of DNA replication in eukaryotic cells and how specific states of gene expression are inherited from one cell generation to the next. In rapidly proliferating cells, such as cancer cells, the process of duplicating the cellular chromosomes begins soon after the chromosomes are separated during mitosis. Previous research has demonstrated that four proteins, the origin recognition complex (ORC), Cdc6, Cdt1, and the minichromosome maintenance $MCM^{(2–7)}$ hexamer proteins, are required for establishing a prereplicative complex (pre-RC) at sites along the chromosomes, many of which are destined to become origins of DNA replication in the S phase of the cell division cycle, the phase at which DNA synthesis occurs. Our studies this year have continued to expand the role of the ORC in inheritance of chromosomes, particularly control of duplication of centrosomes. We have also extended the relationship between DNA replication proteins and other cellular processes such as DNA repair, gene transcription, and assembly of histones.

ORC AND CONTROL OF CENTROSOME DUPLICATION

To maintain genome integrity, DNA and centrosomes must be duplicated exactly once before cell division. Centrosomes are the organelles in cells that organize microtubules (MTs) for many cellular processes, including mitosis. Unlike chromosomes, centrosomes are not known to contain nucleic acid, and thus, how these protein complexes duplicate and how this process is controlled are unclear. Centrosomes contain a core centriole pair that duplicates to two linked pairs that later separate and move to opposite poles before orchestrating the mitotic MT spindle. Centriole pairs are surrounded by pericentriolar material (PCM) that contains numerous proteins. A number of years ago, we demonstrated that the Orc2 subunit was associated with centrosomes, and depletion of Orc2 causes cells to have multiple centrosomes in cells that are arrested in mitosis. It is likely that the multiple centrosomes in these mitotically arrested cells were due to DNA damage caused by progression though S phase in the absence of Orc2 because the chromosomes in Orc2-depleted cells were substantially abnormal and spindles were not attached to kinetochores. We have now demonstrated that another ORC subunit, Orc1, has a critical role in controlling the duplication of centrioles and centrosomes, but in this case, it does so in interphase.

First, by biochemical fractionation, Orc1, Orc2, Orc3, Orc4, and Orc5 subunits were shown to be associated with a centrosome-enriched fraction. Orc1 depletion using RNA interference (RNAi)-based genetics affected disengagement of duplicated centrioles and caused overduplication of centrioles and centrosomes. Orc1 overexpression blocked the centrosome reduplication that occurs during prolonged S-phase arrest in the presence of hydroxyurea, an inhibitor of DNA synthesis. Localization to centrosomes by fusing Orc1 or a fragment of Orc1 that was not capable of promoting DNA replication to a known centrosome localization domain from another protein was sufficient to block hydroxyurea (HU)-induced centrosome and centriole reduplication.

Cyclin E has been shown to be required for the duplication of centrosomes, and overexpression of cyclin E is associated with genome instability and alterations in centrosome numbers in cells. Overexpression of cyclin E, but not cyclin A, overrode the Orc1 control of centrosome reduplication. In contrast, we found that Orc1 localization to centrosomes was promoted by its binding to cyclin A via a sequence in the amino terminus of the Orc1 protein. Cotransfection of Orc1 with cyclin E or cyclin A into human cells and immunoprecipitation of Orc1 showed that both cyclin E and cyclin A were coimmunoprecipitated, but only cyclin A bound directly to Orc1 in vitro. We are currently determining how cyclin E interacts with Orc1. Nevertheless, Orc1 blocked the kinase activity of cyclin A–CDK and cyclin E–CDK protein kinase activity in vitro.

Altogether, our data point to Orc1 as a regulator of cyclin-E-dependent control of centriole and centrosome reduplication. We propose that Orc1 is present in G_1 phase of cells, and once cyclin E has promoted one round of centriole and centrosome duplication, Orc1 blocks its activity. Cyclin E is known to promote the initiation of DNA replication and thus the two events—duplication

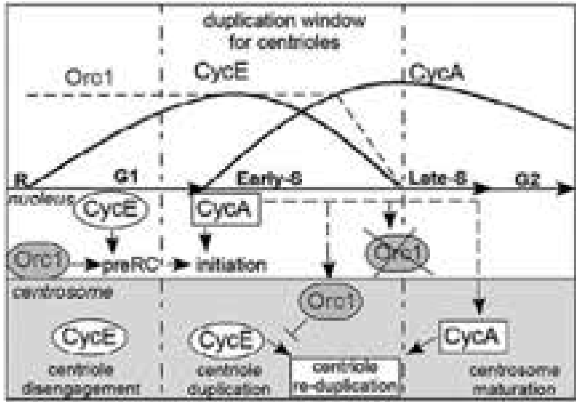

FIGURE 1 Model for the role of Orc1 in control of DNA replication licensing and centriole and centrosome reduplication. As cells pass through the restriction point in G_1 phase and commit to cell division, Orc1 in the nucleus has either already participated in the licensing of DNA replication origins or is acting in cooperation with cyclin E to form pre-RCs at origins. Cyclin E also promotes centriole duplication, possibly by allowing centriole disengagement. If cyclin E persists, then it might promote centriole reduplication. Cyclin E is normally transient, but there is a window of opportunity when cyclin E is still present at centrosomes after normal centriole duplication has taken place. During this time, before cyclin E destruction, Orc1 could block cyclin E function at centrosomes, thereby limiting centriole duplication to once per cell cycle. Orc1 localization to centrosomes could be facilitated by cyclin A promoting nuclear export of Orc1 and localization to centrosomes. Cyclin A also promotes the destruction of the bulk of Orc1 as cells enter S phase, the same time that cyclin E is degraded. Once cyclin E is degraded, the possibility of reduplication is lost because cyclin A cannot induce centriole and centrosome reduplication if the duplicated centrioles are engaged. It is known that cyclin A is involved in centrosome maturation later in the cell cycle. (Reproduced from Hemerly et al. *Science 323:* 789 [2009].)

of chromosomes and control of centrosome copy number—are linked by common regulators (Fig. 1).

CONTROL OF THE INITIATION OF DNA REPLICATION BY THE KINASE DDK

The assembly of a multiprotein pre-RC at each individual origin controls the initiation of DNA replication within chromosomes of eukaryotes. ORC binds to DNA sequences that define the initiation region within chromosomes and recruits a series of proteins, including Cdc6, Cdt1, and MCM proteins that make each origin competent for subsequent initiation of DNA replication. Such competence is established upon mitotic exit or in G_1 phase in rapidly proliferating cells or in G_1 phase in cells stimulated to enter cell division from quiescence.

At least two cell-cycle-regulated protein kinase systems are required for the initiation of DNA replication. The cyclin-dependent protein kinases (CDKs) control entry into S and M phases and activate initiation of DNA replication by phosphorylation of two essential DNA replication factors, Sld2 and Sld3. Studies in the laboratories of H. Araki (National Institute of Genetics, Japan) and J. Diffley (Clare Hall Laboratory, Cancer Research UK) have separately shown that addition of phosphate residues to Sld2 and Sld3 cause them to bind to Dbp11, and the protein complex thus formed allows recruitment of proteins such as Cdc45 to individual origins of DNA replication just before the initiation of DNA replication at that locus. CDKs also contribute to prevention of rereplication in a single S phase by phosphorylation of the pre-RC components ORC, Cdc6, and MCM proteins, but CDKs are not sufficient for the activation of competent pre-RCs on chromosomes. A second cell-cycle-regulated kinase, the Cdc7-Dbf4 kinase (DDK; Dbf4-dependent protein kinase), is also required for initiation at each origin and for monitoring DNA damage as DNA synthesis progresses through S phase.

Our previous studies have shown that DDK phosphorylates multiple subunits of the $MCM^{(2–7)}$ hexamer that is part of the pre-RC and, after activation of the pre-RC, is part of the helicase at each DNA replication fork. We have further shown that loading of Cdc45, a component of the active helicase, requires activation of both S-phase CDK and DDK. More recently, we have shown that the principal and essential DDK substrate for initiation of DNA replication is the Mcm4 subunit of the $MCM^{(2–7)}$ helicase. DDK binds to Mcm4 via a specific docking site, allowing cooperative phosphorylation of multiple sites on an adjacent DDK target sequence. The DDK target sequence lies at the very amino terminus of the Mcm4 protein, in a domain that we call the NSD (*N*-terminal serine/threonine-rich domain), which contains 51 serine residues within a 174-amino-acid unstructured sequence. Complete deletion of the NSD allows cells to grow without the DDK, but the cells grow very poorly. Alternatively, deletion of a shorter region within the NSD allows DDK bypass and better growth of cells. This suggests that the Mcm4 NSD has both positive and inhibitory sequences, and deletion of the inhibitory sequences allows cells to proliferate in the absence of DDK under normal growth conditions. But removal of the NSD inhibitory region in Mcm4 causes cells to become hypersensitive to inhibition of ribonucleotide reductase by HU, suggesting that DDK has a second function that is genetically separable, essential only in the presence of DNA damage in S phase. We suggest that this function is associated with the proposed role for DDK in the intra-S-phase checkpoint that arrests S-phase progression when sufficient DNA damage occurs

during DNA replication. Consistent with this proposed activity, we find that in a strain in which DDK is absent due to the Mcm4 NDS deletion, the checkpoint kinase Rad53 is no longer phosphorylated in the presence of HU. Rad53 phosphorylation is indicative of an activated intra-S-phase checkpoint.

By deleting the Mcm4 NSD inhibitory sequence with altered forms of Sld2 and Sld3 (or Cdc45) that bypass the requirement for CDKs for initiation of DNA replication, we show that cells can now initiate DNA synthesis in G_1 phase in the absence of CDK and DDK. As shown in J. Diffley's laboratory, the requirement of S-CDK for DNA synthesis can be bypassed by using an *sld3-dpb11 fusion* (*SD fusion*) and overexpression of a phosphomimetic *sld2-T84D* mutation from a galactose-inducible promoter. When the altered *mcm4* allele is combined with a *CDC7* deletion in this strain, extensive DNA synthesis occurs in α-factor-arrested cells that do not have active CDK. Alternatively, a strain from H. Araki's laboratory containing a dominant *CDC45* mutation Cdc45^{JET1} that can bypass essential CDK phosphorylation of Sld3 and containing *GAL-sld2-11D* (encoding a galactose-inducible phosphomimetic form of Sld2) can also initiate DNA replication in α-factor-arrested cells when combined with the *mcm4* NSD deletion in the absence of DDK and CDK activity.

By combining CDK and DDK bypass mutations, we have shown that the only essential function of DDK for initiation of DNA replication is to phosphorylate a key region in the amino-terminal region of Mcm4, one of the subunits of the MCM$^{(2-7)}$ hexamer. Thus, the only essential role for DDK is to block an inhibitory sequence within Mcm4. We do not know what this inhibitory region does, but it is possible that it blocks Cdc45 loading onto the MCM complex. The genetically separable function of DDK that is not bypassed by the *MCM4* mutations is only apparent in the presence of DNA damage (Fig. 2).

ROLE OF ABF1 IN NUCLEOTIDE EXCISION REPAIR

Our prior research involved identification of *cis*-acting DNA sequences and the corresponding *trans*-acting proteins that are responsible for the initiation of DNA replication at specific loci in the yeast genome. One such

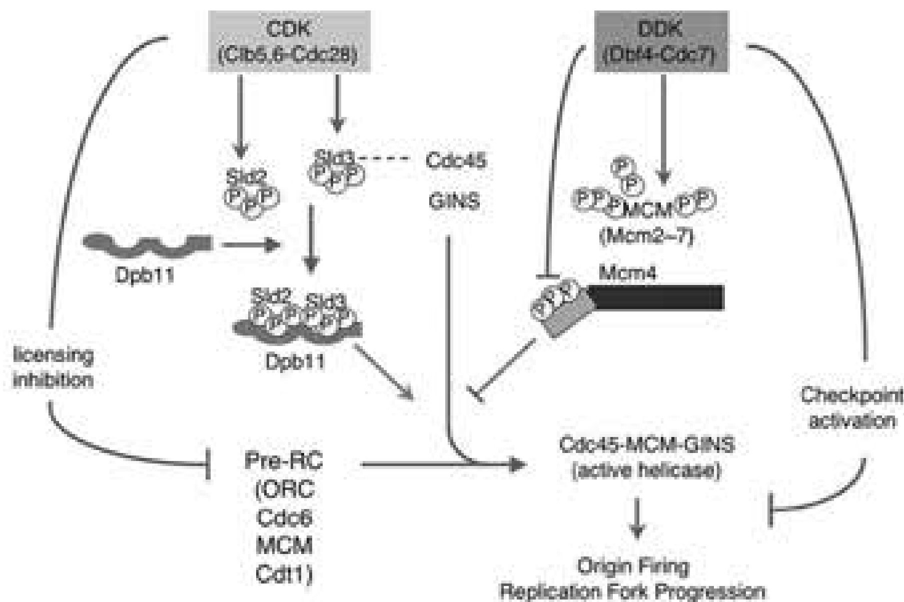

FIGURE 2 A model for coordinated control of initiation of DNA replication. The pre-RC, which consists of ORC, Cdc6, Cdt1, and MCM$^{(2-7)}$, is assembled in G_1 phase of the cell cycle and licenses the initiation of DNA replication in S phase. Activation of the pre-RC occurs by the coordinated action of two protein kinase systems: the S-phase cyclin-dependent kinase (CDK) and the Dbf4-dependent Cdc7 kinase (DDK). Essential CDK targets are Sld2 and Sld3 that then bind Dbp11 (Tanaka et al., *Nature 445:* 328 [2007]; Zegerman and Diffley, *Nature 445:* 281 [2007]). The essential DDK target is the amino terminus of the Mcm4 subunit of the MCM$^{(2-7)}$ complex that eventually becomes part of the helicase with Cdc45 and the GINS proteins. We have shown that DDK blocks an inhibitory activity in the amino terminus of Mcm4. Phosphorylation of this region by DDK blocks inhibitory activity and promotes initiation of DNA replication.

protein, called Abf1 (ARS binding factor 1), was identified as a protein that binds the B3 element of the yeast *ARS1* origin and stimulates the initiation of DNA replication at that locus. In collaboration with Simon Reed's laboratory (Cardiff University, UK), we have shown that the Abf1 protein forms a stable complex with the Rad7 and Rad16 proteins, both of which are required for nucleotide excision repair over the global genome (GG-NER), a repair process that removes DNA damage from nontranscribing DNA. Again, in collaboration with Simon Reed's laboratory, we have now shown that binding of ABF1 to its DNA recognition sequence found at multiple genomic locations promotes efficient GG-NER in yeast. GG-NER adjacent to Abf1-binding sites requires the function of Rad7 and Rad16 proteins.

At the *ARS1* locus, Abf1 contributes with ORC to position nucleosomes at specific positions on either side of the origin of DNA replication, allowing the pre-RC to form in a nucleosome-free region. The latest observations show that mutation of an ABF1-binding site at the *HML*α locus causes loss of ABF1 binding and results in a domain of reduced GG-NER efficiency on one side of the ABF1-binding site. Even though Abf1 binding at this site has been prevented, nucleosome positioning at this site was not altered. Thus, the stimulation of GG-NER adjacent to Abf1-binding sites involves a direct recruitment by Abf1 of proteins to these sites of DNA repair. These studies extend the many activities of Abf1, which include roles in the initiation of DNA replication, transcriptional gene silencing, transcriptional activation of gene expression, and now GG-NER.

INTERACTION BETWEEN PCNA AND THE ELONGATOR COMPLEX

Another protein we discovered as an essential DNA replication and repair protein is the proliferating cell nuclear antigen (PCNA in mammalian cells and Pol30 in yeast *Saccharomyces cerevisiae*). PCNA is a DNA polymerase clamp for DNA polymerase δ, and in addition to tethering this polymerase to the DNA replication fork, it binds numerous other proteins, including chromatin assembly factor-1 (CAF-1), that deposit histones onto two newly replicated or repaired DNA. CAF-1 in turn cooperates with two other histone-binding chaperones, Asf1 and Rtt106, to ensure efficient inheritance of chromatin and stable states of gene expression, such as silenced heterochromatin at silent mating-type genes and at telomeres. The DNA replication or DNA-repair-coupled nucleosome assembly pathway is also important for maintenance of genome stability.

We have identified an interaction between the Elongator histone acetyltransferase protein complex and the DNA replication–coupled nucleosome assembly pathway. Cells lacking Elp3 (K-acetyltransferase Kat9), the catalytic subunit of the six-subunit Elongator complex, partially lose gene silencing at telomeres and at the *HMR* silent-mating-type locus and are sensitive to the DNA-damaging agent HU. Like deletion of the *ELP3*, mutations of each of the four other subunits of the Elongator complex, as well as mutations in Elp3 that compromise the formation of the Elongator complex, also result in loss of silencing and increased sensitivity to DNA-damaging agents. Genetic epistasis analysis indicates that the *elp3*Δ mutant exacerbates the DNA-damage sensitivity of cells lacking histone chaperone Asf1, as well as deletion of the gene encoding the histone H3 lysine 56 (K56) acetyltransferase Rtt109, but the *elp3*Δ deletion did not enhance the phenotype in cells lacking CAF-1. Genetic epistasis experiments with the *elp3*Δ mutant and mutations in specific lysine residues in histone H3 and H4 suggested that Elp3 acts via acetylation of the amino-terminal tail of histone H4. Previous biochemical studies by other investigators suggested that residue K8 in histone H4 is a target of the Elongator complex.

The results suggested that Elp3 functions in the same genetic pathway as CAF-1 to maintain genome stability. Biochemical support for this idea was obtained when we demonstrated an interaction both in vivo and in vitro between the Elongator complex and PCNA. Together, the results have uncovered a new role for the Elongator histone acetyltransferase complex in transcriptional gene silencing and maintenance of genome stability, and it does so in a pathway linked to DNA replication–coupled nucleosome assembly.

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TRANSCRIPTIONAL CONTROL AND THE UBIQUITIN–PROTEASOME SYSTEM

W.P. Tansey A. Chakraborty, G. Collins, A. Daulny, J. Geisinger, F. Geng, A. Leung, D. Khalil, J. Kurland, S. Salghetti, D. Simpson, L. Wang

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

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

REGULATION OF MYC BY UB-MEDIATED PROTEOLYSIS

Myc is a basic helix-loop-helix leucine-zipper transcription factor that features prominently in the control of cell growth. Capable of acting as both a transcriptional activator and repressor, Myc controls the expression of genes required for cell growth and division. Consistent with the type of genes that it regulates, Myc is also is a major human oncoprotein that features in ~70,000 cancer deaths in the United States each year.

Like many transcription factors, particularly those involved in the control of cell growth, Myc is an unstable protein that is destroyed by Ub-mediated proteolysis. Previous work in our laboratory has revealed that the destruction of transcription factors such as Myc can be intimately connected to their ability to activate transcription. Specifically, we have found that the same domain in these proteins that allows them to activate transcription is responsible for their destruction. We have also found that, in some cases, transcription factors *need* to engage the Ub system and become ubiquitylated in order to function. This intimate connection between transcription factor activity and turnover has two important ramifications. First, it predicts that components of the Ub–proteasome system will be directly and intimately involved in the control of gene expression. Second, it reveals that if we study the destruction of transcription factors, we can learn not just about activator proteolysis, but also about activator function. Thus, we believe that by probing the mechanisms through which Myc is destroyed by the Ub system—the elements involved, the cellular players that control Myc turnover, and the pathways that govern Myc stability—we can also gain new insight into how this enigmatic human oncoprotein functions.

For example, we and other investigators have found that adenovirus E1A can stabilize Myc during the course of adenovirus infection. E1A is a prototypical viral oncoprotein that functions to regulate the expression of both viral and cellular genes. The analysis of E1A has been instrumental in revealing critical pathways that lead to cellular transformation. Although the significance of E1A-mediated stabilization of Myc was not known, the intimate connection between transcription factor activity and destruction led us to speculate that this stabilization *must* have a functional consequence. In collaboration with the Hannon and Lowe laboratories here at CSHL, we therefore investigated the mechanism through which E1A stabilizes Myc and probed the relationship of this mechanism to the function of the E1A oncoprotein. We found that 12S E1A, when stably expressed in U2OS cells or in nontransformed IMR90 human diploid fibroblasts, promoted the accumulation of Myc protein. We subsequently showed that E1A expression stabilizes Myc without affecting its ubiquitylation, and that it does so via the transcriptional coactivator p400. This was an interesting finding,

because although the E1A–p400 interaction was known to be important for E1A function, the downstream target of this interaction was unknown. The functional significance of the E1A–p400–Myc nexus was demonstrated by (1) the observation that Myc, like p400, is essential for E1A to induce apoptosis, (2) the finding that E1A can stimulate expression of Myc target genes, and (3) the finding that in vitro transformation defects that arise upon disruption of the p400-binding site in E1A can be ameliorated by overexpression of Myc, arguing that the essential function of this region of E1A is to increase Myc protein levels. Taken together, these data reveal that interaction of E1A with p400 leads to an increase in the levels of p400, which in turn results in a postubiquitylation stabilization of Myc and a subsequent activation of Myc target genes. In essence, E1A (via p400) "hijacks" Myc to perform an important facet of its activities. We are excited to learn about this novel pathway of E1A function and keen to explore the mechanism through which E1A regulates p400 and through which p400 modulates Myc.

THE ROLE OF THE UBIQUITIN–PROTEASOME SYSTEM IN GENE ACTIVITY

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

We are particularly interested in characterizing novel proteins that directly connect the transcription and Ub systems. To this end, we surveyed the literature for examples of proteins with probable links to both systems. This analysis led us to rA9, a mammalian protein that was identified by Dr. Jeff Corden in a two-hybrid screen for factors that bind the carboxy-terminal domain (CTD) of Rpb1, the largest subunit of RNA polymerase II (pol II). rA9 contains a carboxy-terminal domain that binds the CTD (CTD-binding domain, CBD), as well as a series of "SR repeats," commonly found in splicing factors. Interestingly, we observed that rA9 also contains a RING finger and a PHD domain, both of which are associated with Ub ligase activity. The combination of CTD binding and potential E3 activities in rA9 suggested to us that it may function as a Ub ligase within the context of transcription.

To study rA9, we asked whether a related protein is present in the yeast *S. cerevisiae*. Although BLAST searches failed to identify homologous proteins in yeast, we were able to identify sequences that have small, but significant, homology with the CBD in a variety of organisms, including fungi. Within these proteins, the region of CBD homology is located at the carboxyl terminus of the protein, and—remarkably—the majority contain either an amino-terminal RING finger or a RING/PHD combination. We refer to proteins with this architecture as "RC" (*R*ING/*C*BD) or "RPC" (*R*ING/*P*HD/*C*BD) proteins (Fig. 1). The single RPC member we chose to study in *S. cerevisiae* was Asr1, a nonessential protein that has been implicated in the alcohol stress response.

Our studies of Asr1 have found that it is an active Ub ligase that binds directly to pol II via the CTD of Rpb1. We found that interaction of Asr1 with the CTD is dependent on Ser-5 phosphorylation within the CTD, an important observation because this phosphorylation event occurs commensurate with the initiation of transcription. Asr1 thus has the ability to hone in on the population of pol II that is actively engaged in gene expression. Binding of Asr1 to pol II results in the ubiquitylation of at least two subunits in the enzyme, Rpb1 and Rpb2. These ubiquitylation events, in turn, result in the specific ejection of two additional pol II subunits, Rpb4 and Rpb7 (Fig. 2), and are associated with inactivation of polymerase function. The ability of Asr1 to specifically interact with a discrete subset of pol II molecules demonstrates how a component of the Ub–proteasome system can "sense" the modification status of a basal transcription factor and act to alter its subunit composition. Asr1 is thus one of the most explicit examples of how the transcription and Ub systems can intersect. Importantly, our study of Asr1 also demonstrates how ubiquitylation can modulate the composition of a large, multiprotein, complex such as pol II. Ongoing

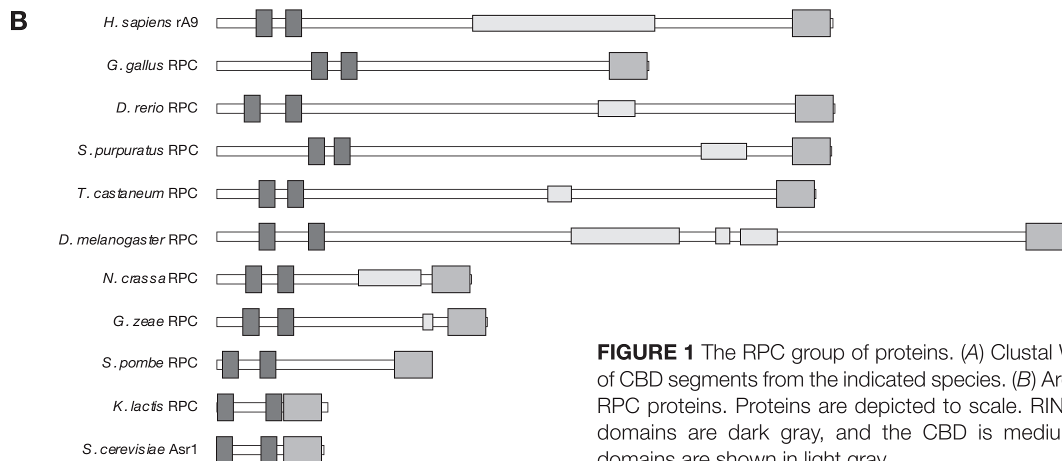

FIGURE 1 The RPC group of proteins. (*A*) Clustal W alignment of CBD segments from the indicated species. (*B*) Architecture of RPC proteins. Proteins are depicted to scale. RING and PHD domains are dark gray, and the CBD is medium gray. RS domains are shown in light gray.

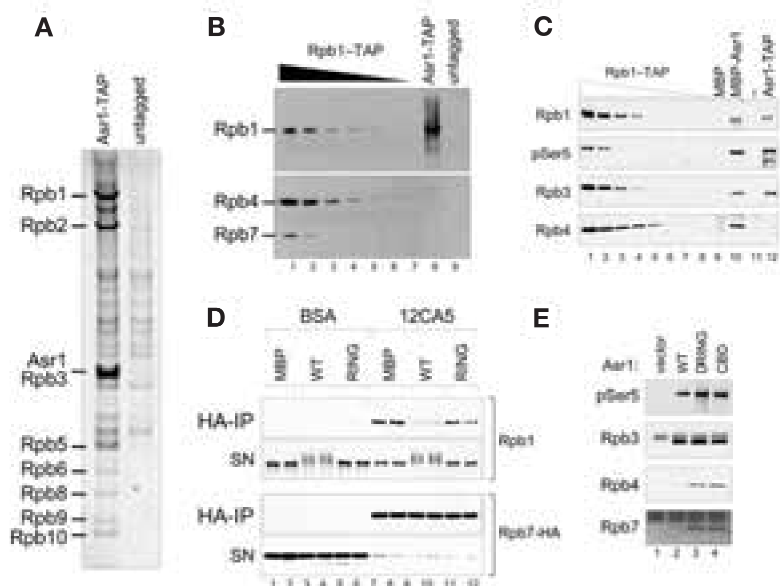

FIGURE 2 Asr1-mediated ubiquitylation excludes Rpb4/7 from the RNA polymerase II (pol II) complex. (*A*) Asr1–TAP purification. TAP purification was performed on extract from either untagged yeast (negative control) or yeast carrying TAP-tagged Asr1. The indicated proteins were identified by mass spectrometry of individual bands and by shotgun analysis in solution. (*B*) The Asr1 complex is devoid of Rpb4/7. Increasing amounts of Rpb1–TAP complexes were run against an Asr1–TAP preparation and probed for Rpb1, Rpb4, and Rpb7 by Western blotting (WB). (*C*) Asr1 can bind pol II that includes Rpb4. Yeast extract was run over an MBP–Asr1 column, and the indicated pol II subunits were detected by WB. Asr1–TAP and Rpb1–TAP complexes are shown for comparison. (*D*) The Ub ligase activity of Asr1 excludes Rpb7 from the pol II complex. Purified pol II complexes were ubiquitylated by Asr1 and Rpb7-HA recovered by immunoprecipitation (IP; BSA is the negative control). Coprecipitating Rpb1 complexes in the IP or supernatant (SN) were detected by WB. (*E*) The RING finger of Asr1 is required to exclude Rpb4 and Rpb7 from the Asr1 complex. Asr1 and the indicated mutants were immunoprecipitated from yeast and probed for pol II subunits by WB.

studies are probing how negative regulation of pol II by these ubiquitylation events impacts control of gene expression in yeast and mammalian cells.

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Galen Collins

CANCER: SIGNAL TRANSDUCTION

Signal Transduction focuses on signaling pathways and cell architecture in normal and cancer cells.

Yuri Lazebnik and colleagues study cell fusion in the context of the hypothesis that viruses and other common human pathogens might cause cancer under certain conditions. They have established that massive chromosomal instability can be engendered by a transient event causing genomic destabilization without permanently affecting mechanisms such as mitosis or proliferation. The agent, in this instance, is an otherwise harmless virus that causes chromosomal disruption by fusing cells whose cell cycle is deregulated by oncogenes. The resulting cells had unique sets of chromosomes and some proved to be capable of producing aggressive epithelial cancers in mice. This year, Lazebnik's lab developed a means of producing hybrid cells more efficiently. Rather than use live viruses to induce fusion, they perfected a method of isolating viral fusogenic proteins. Using these to induce fusion under controlled conditions, they are now exploring the consequences of cell fusion for cell viability and survival.

Changes in tissue architecture are often the first signs of cancer, but very little is known about the genes, proteins, and pathways that regulate cellular shape and polarity. Senthil Muthuswamy has developed a new paradigm for investigating this aspect of cancer biology. Using sophisticated model systems such three-dimensional cell culture platforms and transgenic mice, his team recently found that a protein called Scribble normally coerces breast epithelial cells into the correct organization and shape and enforces resistance to cancer. Scribble is frequently missing in human breast cancer lesions, Muthuswamy's team found, which suggests that it could be an appropriate target for therapies aimed at preventing precancerous lesions from becoming invasive. The lab has also identified new drug targets in patients with tumors that do not respond to chemotherapy. They identified an enzyme called Brk, which is overproduced in patients with HER2-positive tumors and helps them to become resistant to drugs such as Herceptin.

Darryl Pappin's lab develops chemical and computational methods for analysis of proteins and peptides. These constitute fundamental tools for proteomics and can be applied across many fields of biological investigation. Proteins and peptides are typically analyzed via mass spectrometry, a method that involves fragmenting samples by colliding them with gas atoms in a vacuum; masses of the resulting fragments are measured, and computer algorithms match results with known or predicted molecules whose amino acid sequences are either known or inferred. Pappin has developed search engines for mass spectrometry data that enable investigators to sift hundreds of thousands of experimental spectra at a time for database matches. He also seeks to reduce sample complexity via an approach he calls chemical sorting. This includes the use of chelation to enrich phosphopeptides from the total peptide pool and the use of specific affinity-tagged small-molecule inhibitors to segregate classes of kinases or phosphatases for more specific mass spectroscopic analysis.

Jacek Skowronski and colleagues study mechanisms involved in the induction of AIDS by human and simian immunodeficiency viruses (HIV and SIV), focusing on the function of accessory proteins called Nef, Vpr, and Vpx. These virulence factors modify the cellular milieu to disrupt adaptive responses and/or innate antiviral responses and provide an environment conducive for viral replication. This year, Skowronski and colleagues discovered new details about how a simian strain of the AIDS virus replicates in macrophages, a type of immune system cell. The study revealed how Vpx enables efficient reverse transcription in the simian virus and thus overcomes an innate block that otherwise prevents viral replication. This suggests a strategy by which a future drug might interfere with the reproductive machinery of the virus to prevent or limit is ability to spread.

Despite their large variety of genetic abnormalities, cancer cells have been found to be extremely sensitive to the reversal of certain mutations. Raffaella Sordella and colleagues study why cells in certain cancers are responsive to the inhibition of one particular gene or gene product. Why, for instance, do non-small-cell lung cancer cells that have a particular epidermal growth factor (EGF) receptor mutation respond dramatically to its inhibition by the drug Tarceva? This occurs in 15%–20% of patients, the great

majority of whom, within 1–3 years, develop resistance. Various mutations have been implicated in about half of resistant patients. Sordella and colleagues are focusing on as yet unidentified mechanisms of resistance at work in other cases. They have preliminary in vitro data indicating a genetic signature predicting positive response to other treatment modalities. With colleagues at the National Institutes of Health, they are testing this hypothesis in tumor samples taken from relapsed patients.

Nicholas Tonks and colleagues study a family of enzymes called protein tyrosine phosphatases, or PTPs, that remove phosphate groups from other proteins. By changing the phosphorylation state of proteins, PTPs can profoundly affect the health of entire organisms. Tonks' group seeks to characterize fully the PTP family, understand how their activity modifies signaling pathways, and how those pathways are abrogated in serious illnesses, from cancer to Parkinson's disease. The overall goal is to identify new targets and strategies for therapeutic intervention in human disease. This year, the lab made progress in characterizing mice in which the gene encoding JSP-1 (JNK stimulatory phosphatase 1) has been ablated, helping to define the role of this phosphatase in regulating a critical signaling pathway in Parkinson's disease. In collaboration with the Muthuswamy lab at CSHL, they also investigated new roles for PTPs in regulating signaling events in breast cancer and have identified three PTPs as potential novel tumor suppressors.

Several years ago, Lloyd Trotman discovered that the loss of a single copy of a master tumor-suppressing gene called *PTEN* is sufficient to permit tumorigenesis in animal models of prostate cancer. His team later found that complete loss of *PTEN* triggers senescence, a quiescent state that delays or blocks cancer development in affected cells. Recently, the lab has been exploring the impact of varying *PTEN* expression levels, alone and in conjunction with changes in other potent tumor suppressors, including p53, in mouse models for prostate cancer. They have studied how the PTEN protein is transported into and out of the cell nucleus and have sought out specific transport receptor proteins, identifying several strong candidates. They have also worked to validate their cancer progression scheme in human prostate cancer biopsy samples from the Memorial Sloan-Kettering Cancer Center, with the aim of identifying patients who have developed tumors with metastasis-favoring mutations.

Linda Van Aelst's lab studies how aberrations in intracellular signaling involving enzymes called small GTPases can result in disease. They are particularly interested in Ras and Rho GTPases, which help to control cellular growth, differentiation, and morphogenesis. Alterations affecting Ras and Rho function are involved in cancer and various neurodevelopmental disorders. This year, Van Aelst's team extended their study of mutations in a Rho-associated gene called *oligophrenin-1*, determining how they are linked to dysfunctions in the glutamate pathway, specifically to maturation and plasticity of excitatory glutamatergic synapses; they are probing the implications for several illnesses. Separately, they have demonstrated that a GTPase activator called DOCK7, previously shown to have a central role in axonal development and morphology, is important in neuronal migration and may be involved in defects in migration that cause developmental illness.

CELL-TO-CELL FUSION AS A LINK BETWEEN VIRUSES AND CANCER

Y. Lazebnik A. Gottesman

During the last year, we continued to test a hypothesis that we proposed previously. This hypothesis suggests that viruses can contribute to carcinogenesis and tumor progression by fusing cells. Two consequences of such fusion are of particular interest. One is an abnormal combination of properties in a cell that is derived from fusion of cells with distinct properties, such as a transformed cell and a bone marrow stem cell. Another consequence is chromosomal instability, which is a poorly understood consequence of cell fusion and a hallmark of many solid cancers. We previously demonstrated that fusing premalignant cells could produce cells that form aggressive cancers in mice.

This last year, we focused on understanding the rules that determine the fate of cell hybrids, which was done for two reasons. One was our intent to facilitate production of cell hybrids that we needed for a variety of experiments. Another, that the fate of fused cells in the body and even in the dish is surprisingly poorly understood, even though cell hybrids have been implicated in carcinogenesis and are used as a tool of stem cell therapy. One reason for this gap is the lack of an adequate experimental system. Therefore, during the last year, we developed a system in which an increase in ploidy, induced either by cell fusion or by inhibiting cytokinesis, induces apoptosis and, if apoptosis is prevented, inhibits clonogenic survival of the cells. We used this system to outline the pathways that link the ploidy increase to apoptosis and found that this link does not involve the elements implicated previously and is likely to be mediated by yet-to-be characterized pathways. We established that apoptosis in this system can be induced through at least two pathways and we will proceed to dissect them.

Overall, we hope that our research will help to develop an experimental framework to test the role of cell fusion, ploidy increase, and chromosomal instability in cancer.

Yuri Lazebnik

EPITHELIAL CELL BIOLOGY AND CANCER

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We are beginning to get significant insight into genes that are altered in breast cancer, but there is almost a complete lack of understanding of the molecular mechanisms that regulate the development and progression of premalignant lesions. Pathologists use a number of criteria to diagnose and predict prognosis of breast cancer. Salient among them are changes in cell number and changes in cell and tissue architecture. Although we are beginning to understand a lot about the mechanisms that regulate cell proliferation, very little, if anything, is known about the mechanisms that regulate disruption of cell and tissue architecture. The long-term goal of our laboratory is to gain fundamental insights into the mechanisms that regulate changes in cell architecture during the development and progression of premalignant lesions. These studies focus on an unexplored area of cancer biology that is likely to open new avenues for cancer diagnosis and therapy, including identification of biomarkers and strategies for treatment of patients with premalignant and malignant disease.

Epithelial cells within a normal breast are organized into ducts and lobules. Each lobule consists of many individual units referred to as an acinus. Within an acinus is a layer of luminal and myoepithelial cells surrounding an empty lumen. The epithelial cells surrounding this lumen have an asymmetric distribution of membrane proteins where the membrane that is in contact with lumen, referred to as the apical surface, is rich in glycoproteins and microvilli, and the membrane that is in contact with the neighboring cell or the surrounding tissue is rich in cell–cell and cell–matrix junctions and referred to as basolateral surface. This characteristic organization is lost early in premalignant disease and the tissue continues to loose its structure and organization during progression to malignancy. Surprisingly, we know little about what role these changes have during the cancer process and, more importantly, if the changes observed in premalignant lesions are harbingers of what is yet to come.

The molecular mechanisms by which the epithelial cells establish and maintain polarity is an active area of investigation. Current understanding suggests that several proteins and protein complexes function as a highly interactive network during establishment and maintenance of epithelial cell polarity. One such complex, Par6/aPKC/Cdc42, regulates polarization processes during epithelial morphogenesis, astrocyte migration, and axon specification. We find that expression of Par6 in mammary epithelial cells induces epidermal-growth-factor-independent cell proliferation and development of hyperplastic three-dimensional acini without affecting apical-basal polarity. This was dependent on the ability of Par6 to interact with aPKC and Cdc42 but not Lgl and Par3 and its ability to promote sustained activation of MEK/Erk signaling. Interestingly, Par6 is overexpressed in estrogen-receptor-positive primary human breast cancers and in endoplasmic reticulum (ER)-expressing precancerous breast lesions. Thus, deregulation of Par6 in early lesions may provide proliferative lesions, although it may a have a role during cancer progression by cooperating with other oncogenic events. Whether Par6β itself or the pathways it regulates will be excellent candidates for predictive biomarkers or drug targets for premalignant lesions remains to be determined.

We recognize that epithelial cells grown in traditional monolayer cultures on plastic dishes do not have the three-dimensional structural features observed in breast tissue. We reason that activation of oncogenes within the context of the three-dimensional acini is likely to serve as an ideal platform for identifying mechanisms by which oncogenes disrupt cell architecture and acinar structure. We have previously shown that activation of the oncogenic receptor tyrosine kinase ErbB2 within the three-dimensional acinus initiated disruption of cell polarity by disrupting the Par polarity complex. The latter is a major discovery because it identified, for the first time, that oncogenes can directly interact with polarity protein complexes. Inhibition of the interaction between ErbB2 and the Par complex blocked the ability of ErbB2 to disrupt cell polarity, identifying polarity proteins as critical regulators of the oncogenic process. These findings also showed that regulators of normal cell polarity can join the “dark side” and be involved in the biological process that regulates carcinoma.

During the past year, we asked whether changes in polarity proteins function as modulators of cancer by themselves and not just function as downstream effec-

tors of oncogenic signaling. We find that in primary human breast cancers, genes encoding polarity proteins such as Par6 are amplified and overexpressed and, furthermore, that polarity proteins such as Scribble are mislocalized in primary human breast tumors. We discovered that knock down of Scribble in mammary epithelia disrupts cell polarity, blocks three-dimensional morphogenesis, inhibits apoptosis, and induces dysplasia in vivo that progresses to tumors after long latency. Knockdown of Scribble also cooperates with oncogenes such as Myc to transform epithelial cells in three-dimensional acini and induce tumors in vivo by blocking activation of an apoptosis pathway. Like knockdown, mislocalization of Scribble from the cell–cell junction was sufficient to promote cell transformation. Interestingly, spontaneous mammary tumors in mice and humans possess both down-regulated and mislocalized Scribble, suggesting a selection pressure for Scribble inactivation. Thus, we have discovered that Scribble is a novel regulator of breast cancer and that deregulation of the polarity pathways promotes dysplastic and neoplastic growth in mammals by disrupting morphogenesis and inhibiting cell death.

Research in our laboratory thus provides the strongest support to date, by far, for the hypothesis that cell polarity pathways have critical roles during initiation and progression of breast cancer. However, we have only scratched the surface. In addition to the two polarity proteins analyzed, more than 45 genes, and more likely to be identified, are thought to regulate various aspects of cell polarity. Furthermore, the precise nature of the oncogene-induced changes in the cell polarity protein complex remains to be understood. The likelihood of identifying signaling pathways unique to altered polarity proteins is very high because most of the polarity gene products have domain structures consistent with their function as signaling scaffolding molecules.

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CELL SIGNALING IN HIV PATHOGENESIS

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Our interest lies in understanding the molecular mechanisms that underlie the pathogenesis of AIDS and, in particular, understanding the functional consequences of interactions between viral proteins and the regulatory machineries in the infected cells. The main focus of our research is to understand the functions of Nef, Vpr, and Vpx accessory virulence factors of human and simian immunodeficiency viruses (HIV and SIV). We have directed a major effort toward the identification of mechanisms and downstream effectors that mediate the effects of Nef and Vpr in the infected cells, and our experiments have been concentrated in two main areas.

One major focus has been on the isolation and identification of cellular proteins that mediate the effects of Nef on signal transduction and endocytic machineries. This has led to the purification and microsequencing of several cellular factors that associate with Nef in T lymphocytes. Importantly, one of the Nef targets is 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.

The other major focus has been on identification of downstream effectors of lentiviral Vpr and Vpx accessory proteins. These experiments have led to the description of several novel cellular proteins that tightly associate with these factors in hematopoietic cells. Among them are E3 ubiquitin ligase complexes that regulate cell cycle progression and the repair of damaged DNA. The observation that Vpr tightly associates with E3 ubiquitin ligase components is interesting because HIV replication is known to be restricted by protein ubiquitination. Remarkably, we found that both the Vpr and Vpx proteins target a novel substrate adaptor for Cullin 4 and function through their associated Cullin-4 E3 ubiquitin ligase complexes. Here, we describe in more detail our studies of lentival Vpr.

FUNCTIONS OF VPR AND VPX LENTIVIRAL ACCESSORY FACTORS

Vpr and Vpx are multifunctional virion-associated accessory proteins of simian and/or human immunodeficiency viruses (HIV-1, HIV-2, and SIV). One function of HIV-1 Vpr is to mediate translocation of viral reverse transcription complexes into the nucleus in nondividing cells, such as terminally differentiated macrophages. The other is to perturb the cell cycle progression of the infected cell. Both functions are thought to be important for facilitating the HIV-1 life cycle in the infected host. Unlike HIV-1, which possesses only the *vpr* gene, HIV-2 and SIVmac viruses specify both the *vpx* and a closely related *vpx* gene. The Vpr proteins encoded by SIV and HIV-2 block cell cycle progression in the G_2/M phase similar to HIV-1 Vpr but do not have the ability to promote nuclear transport of the preintegration complexes in nondividing cells. Interestingly, Vpx proteins are strictly required for the abilities of viruses of HIV-2/SIVsm lineages to transduce monocyte-derived cells. This probably reflects the ability of Vpx to overcome an as yet uncharacterized block to an early event in the virus life cycle in these cells, but the underlying mechanisms have remained elusive. Because Vpx and Vpr function as adaptor proteins, we thought to identify cellular proteins that they bind, to obtain novel insights into their functions.

VPX AND VPR ASSOCIATE WITH CATALYTIC AND REGULATORY COMPONENTS OF CULLIN-4-BASED E3 UBIQUITIN LIGASE COMPLEXES

We used a combination of biochemical and proteomic approaches to identify cellular proteins associated with human and simian Vpx/Vpr proteins. Briefly, Vpx (or Vpr) and its associated proteins was purified from cells by two sequential immunoprecipitations and analyzed by multidimensional protein identification technology (MudPIT). MudPIT is a combination of chromatographic and mass spectrometric procedures that allow unbiased and sensitive identification of proteins in complex mixtures. These experiments were performed in collaboration with Drs. Michael Washburn, Laurence Florens, and Selene Swanson of Stowers Institute for Medical Research (Kansas City, Kansas).

The MudPIT analysis identified three relatively abundant polypeptides—DDB1, DDA1, and VprBP/DCAF1—that were specifically and abundantly associated with SIVmac Vpx and with HIV-1 and SIVmac Vpr

proteins. DDB1 is an obligatory subunit of all known E3 ligase complexes assembled on a Cullin-4 (Cul4) scaffold. VprBP/DCAF1, a known HIV-1 Vpr-binding protein recently shown to bind DDB1, is thought to function as one of many substrate receptors that recruit cellular proteins for ubiquitination by the E3 enzyme. DDA1 was also shown to bind DDB1 and has been implicated for regulation of E3 ligase catalytic activity. Thus, our findings tentatively linked Vpx to Cul4 RING E3 (Cul4 E3) ubiquitin ligases.

Our subsequent biochemical studies revealed that DDA1, DDB1, and VprBP/DCAF1 form a ternary complex, which is then targeted by Vpx, and that this complex links Vpx proteins to the catalytic core of the ubiquitin ligase organized on the Cul4 scaffold. The notion that Vpx proteins target a Cul4-based E3 ubiquitin ligase is interesting because previous evidence indicated that replication of primate lentiviruses is restricted to some extent by protein ubiquitination. These findings raised the possibility that Vpx/Vpr promotes lentivirus replication by modulating protein ubiquitination in infected cells.

VPX USURPS VPRBP/DCAF1 TO ENABLE MACOPHAGE TRANSDUCTION BY SIVMAC

The ability of Vpx to enable infection of primary macrophages is well documented, yet the immediate downstream mediator(s) of Vpx remains unknown. Therefore, experiments were performed to assess whether the interaction with VprBP/DCAF1 and its associated E3 complex is important for Vpx's ability to facilitate macrophage transduction by SIVmac 239. Because this function is probably mediated by the virion-bound Vpx molecules, we first identified Vpx amino acid substitutions that disrupt binding to VprBP/DCAF1 without negatively affecting Vpx incorporation into SIVmac 239 virions. Next, we measured the abilities of virions containing wild-type or VprBP/DCAF1 binding-deficient Vpx proteins to transduce human monocyte-derived adherent macrophages. We found that wild-type Vpx stimulated macrophage transduction by up to 100-fold, whereas Vpx failed to support macrophage infection, even though the mutant Vpx molecules were efficiently incorporated into the virions. In contrast, both viruses displayed similar infectivities to primary $CD4^+$ T lymphocytes and Jurkat T cells, indicating that Vpx is required specifically for transduction of primary macrophages. Subsequent experiments using HIV-2 Vpx yielded similar results. These findings link the ability of HIV-2/SIVmac Vpx to enhance macrophage transduction to its interaction with VprBP/DCAF1 and its associated E3 ubiquitin ligase complex.

VPX USES VPRBP/DCAF1 TO SUPPORT REVERSE TRANSCRIPTION OF SIVMAC IN MACROPHAGES

To assess at which stage in the lentivirus life cycle the replication of Vpx-deficient virus is arrested, we examined the effect of VprBP/DCAF1-binding-defective Vpx proteins on reverse transcription (RT) of the incoming SIVmac genomes. Interestingly, we found that RT was defective following infection with virions lacking, or containing mutant, Vpx proteins. Specifically, in these cases, the steady-state levels of the early strong-stop RTs, U3, gag, and late RTs were approximately tenfold to 1000-fold lower compared to infections with virions containing wild-type Vpx. Importantly, Vpx was not required for efficient RT following infection of Jurkat T cells. Together, these observations indicate that Vpx is required for events that lead to an efficient initiation and progression of RT of the SIVmac genome in macrophages and thus define a new function for lentiviral Vpx.

VPRBP/DCAF1 MEDIATES MACROPHAGE TRANSDUCTION BY SIVMAC

To obtain further insight into the role of VprBP/DCAF1, we knocked down its expression in macrophages by RNA interference (RNAi). We found that nontargeting, negative control, small interfering RNAs (siRNA) decreased transduction efficiencies by only approximately 30%, and the magnitude of this effect was constant over a wide range of siRNA concentrations. These observations indicate that nonspecific engagement of RNAi machinery had only a minor negative effect on macrophage transduction by SIVmac 239. In contrast, RNAi to VprBP/DCAF1 decreased transduction efficiency by approximately tenfold to 100-fold, in a dose-dependent manner. As expected, the inefficient transduction was associated with a very inefficient reverse transcription of the incoming viral RNA. Of note, VprBP/DCAF1 depletion in U2OS cells did not compromise transduction of these cells by the SIVmac virus regardless of the presence or absence of Vpx. Together, these data indicate that VprBP/DCAF1 is required for efficient RT of lentiviruses possessing Vpx in primary macrophages.

TENTATIVE MODELS FOR THE VPX FUNCTION

Our findings support a model in which Vpx usurps the Cul4 E3 ubiquitin ligase using the VprBP/DCAF1 to overcome a block to lentivirus replication upon its entry into monocyte-derived cells. How does Vpx facilitate RT

in macrophages via its interaction with VprBP/DCAF1? We initially considered that the VprBP-linked Cul4 E3 complex restricts SIVmac infection in macrophages and that Vpx counters the restriction by inhibiting catalytic activity of this E3. However, our data from RNAi experiments reveal that VprBP/DCAF1 is not required for the restriction to occur and, therefore, do not support this possibility. Furthermore, the incoming virions probably contain at most only several hundred Vpx molecules, similar to Vpr, which is also virion recruited through its interaction with Gag p6. Therefore, it is difficult to envision that the limited amounts of virion-bound Vpx would be able to saturate and inhibit the cellular pool of VprBP/DCAF1-associated Cul4 E3 complexes, even by a noncompetitive mechanism.

Our evidence indicates that instead of blocking SIVmac replication, VprBP/DCAF1 is required for Vpx to overcome the block, implying that Vpx uses VprBP/DCAF1-associated E3 to enable RT in macrophages. Notably, the same VprBP/DCAF1-associated ubiquitin ligase is targeted by a Vpx paralog Vpr that stimulates the intrinsic catalytic activity of this E3. Our findings that both Vpx and Vpr interact with VprBP/DCAF1 in a similar manner suggest that Vpx also usurps the VprBP/DCAF1-associated E3, probably to inactivate a cellular factor that inhibits lentivirus replication in macrophages. Indeed, viral accessory proteins are known to use E3 ubiquitin ligases to direct ubiquitination and proteasomal degradation of cellular proteins that mediate innate immunity to viral infection. Our ongoing experiments are aimed to identify cellular proteins whose ubiquitination is altered by Vpx. We expect that our findings will advance the understanding of this important virulence factor.

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MOLECULAR TARGETED THERAPY OF LUNG CANCER: EGFR MUTATIONS AND RESPONSE OF EGFR INHIBITORS

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Despite considerable progress achieved in the management of lung cancer, it remains one of the leading causes of death worldwide, with an estimated 215,020 new cases and 161,840 deaths in 2008 and an overall 5-year mortality rate of 85% (American Cancer Society, 2008). Among all the different histological types, non-small-cell lung cancer (NSCLC) is the most common type, comprising 75%–80% of all lung cancers.

Although surgical resection has been shown to be a highly effective treatment for NSCLC, the majority of patients are diagnosed with regionally advanced or metastatic disease, thus limiting therapeutic options to chemotherapy. To date, the mainstay of treatment for NSCLC is usually based on cytotoxic agents such as taxane, doxorubicin, cyclophosphoamide, and platinum-based compounds. Unfortunately, these regimens have been shown to have very limited activity, and they are almost always associated with high toxicity and severe adverse effects.

In recent years, rapid advances in our understanding of the molecular mechanisms of tumor onset and progression have led to the development of new and more effective treatments (i.e., molecular targeted therapies) that in some instances have dramatically improved the outcome of certain cancers (e.g., imatinib in the treatment of chronic myelogenous leukemia and gastrointestinal cancers, and tamoxifen and Herceptin for breast cancer). Because of their usually very limited side effects, they also considerably impact the quality of life of patients. Specifically in the case of lung cancer, much excitement has recently been generated by the finding that a group of NSCLC patients benefited greatly from treatment with selective epidermal growth factor receptor (EGFR) inhibitors.

EGFR is a member of the Erb family of membrane receptors, which are important mediators of cell growth, differentiation, and survival. It has been shown to have a pivotal role in the development and progression of many epithelial cancers, including NSCLC, and thus, not surprisingly, has been the focus of intense efforts to develop new targeted therapies. The EGFR is a 170-kDa transmembrane glycoprotein that consists of an extracellular moiety that recognizes and binds to specific ligands: a hydrophobic *trans*-membrane domain involved in the interaction of the receptor with the cell membrane and an intracellular domain that contains tyrosine kinase enzymatic activity. Upon ligand binding, EGFRs form homodimers or heterodimers with other Erb family members, activating their intrinsic kinase activity and resulting in the dynamic modification—with phosphates added and removed at various rates—of several substrates, including tyrosine residues located in their cytoplasmic tails. These phosphotyrosine residues can then engage specific cytosolic signaling proteins, leading to the activation of different signaling pathways (e.g., MAPK, PI3K, STAT3/5, PLC-γ) that determine the biological activity of EGFR in a given cell type.

Currently, there are two major classes of anti-EGFR agents: monoclonal antibodies (mAbs) directed against the extracellular domain of the EGFR and low-molecular-weight tyrosine kinase inhibitors (TKIs) that inhibit the tyrosine kinase domain of the receptor generally by competing with ATP for the ATP-binding site. The mechanism of action and the biological effects of mAbs and TKIs differ, leading to diverse clinical outcomes. To date, gefitinib (Iressa) and erlotinib (Tarceva)—two selective EGFR inhibitors with similar chemical features—have been demonstrated to have the most robust effects in the treatment of NSCLC. Overall current clinical trials of erlotinib in NSCLC showed an objective response rate of 22% and an overall rate of nonprogression at 6 weeks of 52.8%. Interestingly, responses are more commonly observed in patients of Asian ethnicity, in women, in patients with bronchoalveolar adenocarcinoma, and in nonsmokers.

In 2004, we showed that the majority of tumors that dramatically responded to gefitinib/erlotinib treatment harbored previously uncharacterized mutations within the EGFR locus. We as well as other groups were able to determine that a large majority of EGFR mutations affect its kinase domain. Specifically, they comprise small in-frame deletions around codons 746–750 (54%) and missense mutations that lead to a substitution of leucine for arginine at codon 858 (45%). However, these

mutations are rare: They are found in ~15% of unselected NSCLC patients of Caucasian/African origin and in 40% of Asian patients. These mutations are also practically absent in all other types of cancer, with the exception of cholangiocarcinoma in which they occur with a frequency similar to that observed in lung cancer.

At the molecular level, EGFR mutations dramatically change the biochemical properties of the receptor. We demonstrated that they increase the sensitivity of tumor cells to gefitinib in part by increasingly activating antiapoptotic pathways (i.e., AKT and STAT) on which tumor cells become dependent.

Although patients with EGFR mutations often have impressive and durable clinical responses, they almost invariably develop resistance, usually within 12 months. The first mechanism of resistance to be discovered was the acquisition of an additional mutation in exon 20 of EGFR, resulting in a substitution of threonine for methionine at position 790. T790M mutations have been identified in ~30% of cases of acquired resistance to EGFR TKI. The 790 residue is often referred as the "gatekeeper" residue in tyrosine kinases and is analogous to resistant mutations in ABL and KIT kinases observed in cases of imatinib resistance. At a molecular level, it has been proposed that the presence of a methionine at position 790 changes the conformation of the receptor, altering the EGFR affinity to selective TKI. The discovery of this secondary mutation has spurred the pharmaceutical industry to develop second-generation EGFR inhibitors.

In addition to secondary mutations in the same EGFR, amplification of c-MET has recently been identified as another potential acquired resistance mechanism. In a preliminary study, 22% (4/18) of cases of NSCLC with acquired resistance to gefitinib/erlotinib have been shown to harbor c-MET amplification. Interestingly, some of these tumors contained both the T790M mutation and c-MET amplification. Although the role of c-MET amplification in EGFR TKI-acquired resistance has yet to be definitively proven, current studies suggest that the presence of T790M and c-MET amplification could account for up to ~40% of all cases. Thus, despite considerable progress in identifying acquired resistance mechanisms, new determinants of EGFR TKI have yet to be uncovered in the majority of tumors that have relapsed.

The studies conducted in our laboratory are aimed at gaining an understanding of how and why cancer cells become dependent on mutant EGFR for their survival and how, in instances of acquired resistance, these same cells become refractory to EGFR selective inhibition.

Functional Analysis of Mutant EGFR Signaling Pathways

T. Linsted, M. Pineda

One of the main goals of our studies is to understand why inhibition of EGFR results in cancer cell death (oncogene addiction). Several theories have been proposed to provide a molecular explanation to cancer cell dependency on the activity of certain oncogenes. It has been hypothesized that the presence of selective oncogenes (e.g., mutant EGFR) can lead to a rearrangement of preexisting cellular signaling networks, resulting in an increased vulnerability to apoptotic stimuli. More recently, our colleague Dr. Jeff Settleman at Massachusetts General Hospital proposed an alternative theory termed "oncogenic shock." On the basis of this theory, oncogenes activate proapoptotic and antiapoptotic signaling pathways characterized by different kinetics of inactivation. As a consequence of oncogene inactivation, long-lived proapoptotic pathways will not be effectively balanced by prosurvival short-lived signaling pathways. This imbalance in signaling supposedly results in cancer cell death.

Interestingly, we and other investigators have shown that mutant EGFRs are characterized by an augmented kinase activity and an increased activation of AKT and STAT prosurvival pathways. We are currently studying the molecular mechanism underlying these qualitative signaling differences and investigating whether and how these same signaling pathways play a part in determining cell addiction to mutant EGFR.

Identification of New Determinants of Erlotinib and Gefitinib Sensitivity

S. Fenoglio, Z. Yao

Retrospective studies have showed a high degree of correlation between the presence of EGFR somatic mutations and responses to erlotinib and gefitinib treatment. At the same time, they also indicated that the length and extent of the response to gefitinib and erlotinib treatment are highly variable, often lasting only 6 months and occasionally more than 4 years. The presence of secondary mutations within the EGFR locus and amplification of the c-*met* gene have been specifically suggested to be important mechanisms of acquired resistance. We are interested in uncovering new determinants of the cellular response to EGFR inhibition with the goal of developing new therapeutic options for NSCLC treatment.

To this aim, we developed an in vitro cell-based system that led us to the identification of SOCS2 as a potential player in gefitinib and erlotinib primary and acquired resistance. We are currently investigating the molecular mechanism underlying the increased resistance observed in cells overexpressing SOCS2.

Interestingly, we showed that SOCS2 expression is regulated in NSCLC by estrogen receptor activity and that, in the context of NSCLC, estrogen antagonizes the oncogenic potential of mutant EGFR by regulating the expression of SOCS2. On the basis of these data, we are investigating whether low levels of estrogen receptor activity and SOCS2 are required for the establishment of tumors driven by mutant EGFR. These findings could provide new therapeutic options and potentially explain the observation that in postmenopausal women, in whom levels of estrogen are particularly low, lung tumors containing mutant EGFR are more common.

Are We Targeting the Right Cellular Compartment?

M. Camiolo

We cannot exclude at this time the possibility that the presence of a subpopulation of cells that are not dependent on EGFR for growth or survival could be an alternative hypothesis to explain tumor relapse upon erlotinib or gefitinib treatment. Recently, in fact, in many tumors (i.e., glioblastoma, breast cancer, chronic myelogenous leukemia), it has been shown that a subpopulation of cells displays stem cell characteristics and, interestingly, more resistance to chemotherapy.

We are currently investigating whether cells with stem cell properties are present in NSCLC tumors driven by mutant EGFR and whether these same cells are sensitive to EGFR inhibition.

Identification of New NSCLC Therapeutic Targets

M. Pineda, S. Fenoglio, H. Archibald

On the basis of the successes of gefitinib and erlotinib, our laboratory is interested in uncovering new oncogenes that, similar to EGFR, can be used as therapeutic targets for the treatment of NSCLC. To this aim, we are using functional genomic screens to uncover signaling pathways and genes on which NSCLC cells rely for their survival.

The experience with gefitinib and erlotinib has taught us that in some cases, molecular-targeted therapies work in only a small subset of patients. The low frequency of genetically defined responsive patient subsets thus suggests that it will be necessary to conduct a broader sampling to achieve a representation of genetic diversity. For example, to detect a response similar to that observed in the case of gefitinib and erlotinib treatment in NSCLC patients (i.e., 10%), we estimate that a cell-based screen will require a minimum of 50 cell lines.

In the past year, we collected more than 80 NSCLC-derived cell lines. By using a compound-based screen, we were able to classify the cell lines of our in-house collection in several clusters characterized by different sensitivities to a large pool of compounds targeting signaling pathways known to be deregulated in tumors. We were successful in identifying a subset of cell lines that display high sensitivity to platelet-derived growth factor receptor (PDGFR)-selective inhibitors, and we are currently investigating the molecular basis of their selective sensitivity. Although the benefit of a PDGFR-based therapy in the treatment of NSCLC has yet to be elucidated, we are attempting, in collaboration with Dr. Scott Powers' group here at CSHL, to identify genetic markers that can be used in the clinic for the up-front selection of tumors that will respond to PDGFR-selective inhibition.

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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 coordinated 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. Overall, the objective of the lab is to develop tools for analysis of PTP regulation and function and integrate them with state-of-the-art cell and animal models, to define critical tyrosine phosphorylation-dependent signaling events in human disease. It is anticipated that these studies will lead to identification of novel therapeutic targets and biomarkers at two levels. By elucidating the signaling function of PTPs in disease models, we will reveal examples in which the PTPs themselves, or regulators of PTP function, are targets. Furthermore, we will use the PTPs as probes to define critical signaling pathways in human disease from which novel targets will be identified.

There was significant turnover in the lab during the last year. Seung Jun Kim completed his stay as a Visiting Scientist and returned to the Korea Research Institute of Bioscience and Biotechnology at the University of Science and Technology in Korea. Navasona Krishnan joined us as a postdoctoral fellow, having completed his Ph.D. in the lab of Donald Becker at the University of Nebraska, Lincoln. In addition, two graduate students from Stony Brook began their Ph.D. studies: Mathangi Ramesh is a member of the Molecular and Cellular Biology Program and Ming Yang is a member of the Biochemistry and Structural Biology Program. Finally, Xin Cheng joined us as a Research Associate.

FUNCTIONAL ANALYSIS OF THE "PTPOME"

We have now identified all of the PTP genes in the human genome (there are ~100) and various studies from many labs have pointed to the functional importance of PTPs in the control of cell signaling. It is now apparent that they have the capacity to function both positively and negatively in the regulation of signal transduction. Furthermore, PTPs have the potential to display exquisite substrate and functional specificity in vivo. Nevertheless, the majority of members of the PTP superfamily have yet to be characterized. Hence, the PTPs remain a largely untapped resource that can be exploited to reveal new insights into the regulation of signal transduction. Having defined the composition of the PTP superfamily, the overall goal of this aspect of the work in the lab is to develop the tools and reagents for a functional analysis of the "PTPome," i.e., to analyze the function of members of the PTP superfamily, under normal and pathophysiological conditions, from the perspective of the family as a whole. We have constructed a library of short hairpin RNAs (shRNAs) that targets each of the PTPs (five shRNAs for each PTP). This will allow us to interrogate systematically the function of these signaling enzymes by RNA interference (RNAi) in various disease models. In addition, we have been assembling a collection of antibodies to each of the PTPs, to facilitate analysis of protein suppression by RNAi, and a collection of expression plasmids for each of the PTPs, to facilitate follow-up studies on candidates identified in RNAi screens.

Our first application of this library is being conducted in collaboration with Senthil Muthuswamy here at CSHL, who is an expert in cell models of breast cancer. HER2, a member of the epidermal growth factor receptor (EGFR) family of PTKs, is overexpressed in ~25% of breast cancer patients, which correlates with poor prognosis and high invasiveness. To study systematically the roles of the PTP family in regulating HER2 signaling, in particular their influence on mammary epithelial cell motility and invasiveness, we conducted a loss-of-

function screen using the PTP shRNA library. MCF10A cells that were engineered to express a HER2-FKBP fusion protein, the activity of which can be induced with the small molecule dimerizer AP1510, were used in the screen. This screen identified shRNAs to three PTPs (PTPRG, PTPRR, and PTPN23) that enhanced motility of MCF10A cells expressing HER2. PTPRR-directed shRNAs enhanced cell motility in a HER2-dependent manner, whereas shRNAs to both PTPRG and PTPN23 enhanced motility in either the presence or absence of HER2 signaling. Interestingly, further investigation showed that only PTPN23 shRNA induced invasion when cells were grown on a Matrigel/collagen mixture. However, although PTPN23 shRNA itself was sufficient to enhance invasiveness, activation of HER2 signaling accelerated the effects, indicating cooperation between suppression of PTPN23 and activation of HER2. We are currently identifying the critical PTP substrates that underlie these effects. Although the genes for PTPRG and PTPN23 are located at chromosome 3p21, a hot spot for deletions in various cancers, this is the first indication of potential roles in breast cancer.

In addition to these PTPs that may play a tumor-suppressor function, there are PTPs that may play a positive role in HER2 signaling. Although PTP1B plays a well-established role in down-regulating insulin and leptin signaling, its function is not restricted to metabolic regulation. The *PTP1B* gene is located at chromosome 20q13, which is a region that is frequently amplified in breast cancer and associated with poor prognosis. Studies from the Tremblay (McGill University, Montreal) and Neel (Beth Israel Deaconess Medical Center, Harvard Medical School) labs have shown that when mice expressing activated alleles of HER2 were crossed with *PTP1B* knockout mice, tumor development was delayed and the incidence of lung metastases was decreased. These observations suggest that PTP1B also plays a *positive* role in regulating signaling events associated with breast tumorigenesis. With this in mind, we tested the effects of suppressing PTP1B expression by RNAi on migration of MCF10A cells expressing HER2 in the presence or absence of the activating ligand. Consistent with such a positive function in the regulation of HER2 signaling, we observed that suppression of PTP1B levels by ~60% attenuated HER2-induced migration. The PTP1B protein has a long half-life, and difficulties in establishing full suppression by RNAi are well known. Nonetheless, the effect on migration was striking, even with only partial suppression of PTP1B. Interestingly, suppression of the closely related enzyme TCPTP was without effect. In addition, we noted that shRNAs to some dual specificity phosphatases (DSPs) and myotubularins were inhibitory to HER2-induced migration. These are of interest because if they are shown to be positive regulators of HER2 signaling, these PTP family members may themselves prove to be therapeutic targets. Overall, this approach shows great potential for shedding new light on the roles of PTPs in the etiology of breast cancer.

REGULATION OF PTP FUNCTION BY REVERSIBLE OXIDATION

The signature motif of the PTP family, [I/V]-HCxxGxxR[S/T], contains an invariant Cys residue, which, due to the unique environment of the active site, is characterized by an extremely low p$K\alpha$. This promotes the function of this Cys residue as a nucleophile in catalysis, but renders it highly susceptible to oxidation, with concomitant abrogation of nucleophilic function and inhibition of PTP activity. Work from several labs, including this one, has now established that multiple PTPs are transiently oxidized and inactivated in response to a wide array of cellular stimuli, which represents a novel tier of control of tyrosine-phosphorylation-dependent signaling. Hence, the ability to detect reversible oxidation of PTPs in vivo is critical to understanding the complex biological role of Reactive Oxygen Species (ROS) in the control of cellular signaling. This year, we published a new assay to measure reversible PTP oxidation in cells. The strategy involves labeling the active-site Cys residue in those PTPs that undergo stimulus-induced reversible oxidation with biotinylated small molecules. The advantage of this approach over those already in use is that we can now detect oxidation of all the major subtypes from within the PTP family, in particular receptor PTPs. We are currently working on several collaborations in order to test our assays in as broad a range of circumstances as possible.

Identification of an oxidation-induced cyclic sulphenamide modification at the active site of PTP1B and the generation of antibodies to target the oxidized conformation of the PTP. In order to explore mechanisms by which specificity in PTP oxidation may be achieved, we set out to develop reagents that would recognize selectively the oxidized form of PTP1B, which is the prototypic member of the PTP family and has important roles in down-regulation of various PTK-induced signaling pathways, including insulin signaling. Previously, in collaboration with David Barford (ICR London), we had used X-ray crystallography to examine the structural consequences of oxidation on the active site of PTP1B. We noted that after oxidation of the catalytic Cys to sulphenic acid, there was a rapid

condensation reaction, which resulted in formation of a unique cyclic sulphenamide species. The consequences for the architecture of the active site are profound. Upon formation of the cyclic sulphenamide, critical hydrogen bonds are broken, which triggers residues from the active site to adopt solvent-exposed positions in a process that was readily reversible upon incubation of the crystals with a reducing agent. We have shown that insulin induces the transient oxidation and inactivation of PTP1B (and TCPTP) and that this suppression of PTP function by oxidation is important for an optimal signaling response in insulin-responsive cells. Considering this, and the structural consequences of reversible oxidation, we set out to test the hypothesis that stabilization of this inactive conformation (PTP1B-OX), which would be formed following insulin-induced production of ROS, would augment the hormone response by removing an inhibitory constraint on signaling.

Our approach was to attempt to generate conformation-specific antibodies that recognize oxidation-specific epitopes in PTP1B-OX that would not be found in the active, reduced enzyme. An important breakthrough was our discovery that a double-point mutation at the active site of PTP1B induced a stable conformation that is indistinguishable in structure from the PTP1B-OX conformation induced by H_2O_2, and this could therefore be used as an antigen. We immunized chickens with purified PTP1B-OX mutant protein and harvested spleen and bone marrow from immunized animals in which we had detected a robust increase in serum antibody titer to the purified antigen. We chose to adopt the phage display technology, which allows rapid and efficient screening of libraries of high complexity. Antibody molecules contain discrete domains that can be produced by recombinant techniques. To generate the library, we used the small Fv (fragment variable), which is composed of the variable-light (V_L) and variable-heavy (V_H) regions. In a single-chain variable fragment (scFv), the two variable regions are artificially joined with a neutral peptide linker. The recombinant antibody scFvs are presented on the surface of bacteriophage and can be selected from combinatorial libraries in vitro. We used a subtractive panning strategy. PTP1B-OX mutant protein biotinylated at the amino terminus was mixed with the library under reducing conditions in the presence of a ~50-fold molar excess of wild-type enzyme. The PTP1B-OX–scFv-phage complex from this mixture was isolated on streptavidin beads and phage particles with surface-exposed scFvs bound to PTP1B-OX were then eluted and amplified. A total of four rounds of panning were performed to enrich the library with pools of antibodies specific to the mutant form of the enzyme. From these pools of scFvs, we have isolated 115 distinct (by sequence of their hypervariable regions) candidates that we are now testing.

Our expectation was that scFvs that are able to bind to and stabilize the oxidized conformation of PTP1B in solution would inhibit its reactivation by reducing agent, but they would have no direct inhibitory effect on phosphatase activity of the reduced enzyme in assays in vitro. We established conditions under which wild-type PTP1B could be reversibly oxidized in vitro. PTP1B was inactivated following addition of H_2O_2; however, phosphatase activity was completely restored upon the removal of H_2O_2 by a quick buffer exchange and addition of reducing agent. Purified, bacterially expressed scFvs were incubated with PTP1B after H_2O_2 treatment and the ability of an individual scFv to stabilize the reversibly oxidized, inactive conformation of the PTP was assessed by the ability of the antibody to inhibit the reactivation of the enzyme by reducing agent. Thus far, we have screened ~25% of the scFv candidates, with extremely encouraging results. We have found four scFvs that showed significant inhibition of the reactivation of PTP1B-OX by reducing agent, but did not exert any direct inhibitory effect on activity. In order to validate this approach further, we tested the effects of expressing one of these as a single-chain antibody fragment “intrabody” to PTP1B, using 293T cells as a convenient expression system. We expressed this intrabody transiently and then tested for effects on insulin signaling, focusing initially on the tyrosine phosphorylation status of the β-subunit of the insulin receptor and IRS-1. Initial indications are that for both substrates, expression of the intrabody had no impact on the basal level of tyrosyl phosphorylation, but it enhanced and extended the time course of insulin-induced phosphorylation, consistent with our proposed mechanism of action. We are currently completing the screen and testing further for effects on insulin signaling.

Quite apart from their significance as reagents, these antibodies may influence strategies for development of PTP1B-directed therapeutics. The phenotype of the knockout mouse, together with structural and biochemical data from various groups, has established PTP1B as a key regulator of insulin and leptin signaling. Consequently, it became a highly prized target in the pharmaceutical industry for therapeutic intervention in diabetes and obesity. Although there have been major programs in industry focused on developing small-molecule inhibitors of PTP1B, these efforts have been frustrated by technical challenges arising from the chemical properties of the PTP active site. The susceptibility of PTPs to oxidation causes problems in high-throughput screens. In addition, the tendency of potent inhibitors to

be highly charged, such as nonhydrolyzable pTyr mimetics, presents problems with respect to bioavailability. Consequently, new approaches to inhibition of PTP1B are required to reinvigorate drug development efforts. These redox-dependent changes in conformation suggest a novel way to think about inhibiting PTPs in a therapeutic context. Reversible oxidation of PTP1B generates a form of the enzyme in which the problematic chemical properties of the enzyme are circumvented and new binding surfaces for small-molecule inhibitors are presented. Therefore, if it is possible to stabilize the oxidized, inactive form of PTP1B with an appropriate therapeutic molecule that mimics the effects of these antibodies, this could then provide a new strategy for PTP-directed drug development that may circumvent the difficulties that are faced when targeting the PTP active site with highly charged inhibitors. Stabilization of the inactive PTP1B-OX conformation may potentiate insulin signaling in a manner similar to inhibiting the catalytically active form of the enzyme, analogous to stabilization of the inactive form of p210 BCR-ABL by Gleevec. In addition, if one assumes that in responding to insulin, the cell targets for oxidation the pool of PTP1B that is important for regulation of the signaling response, then this strategy will also target that pool specifically, possibly also reducing complications of side effects that may accompany inhibition of the native enzyme as a whole. Other PTPs, in addition to PTP1B, have also been shown to form a cyclic sulphenamide upon oxidation, suggesting that this approach may offer a general strategy for inhibiting this enzyme family.

FUNCTIONAL ANALYSIS OF JSP1

Previously, we identified JNK stimulatory phosphatase 1 (JSP1) as a DSP that has the capacity to activate the JNK mitogen-activated protein kinase (MAPK) specifically, exerting its effects upstream of the MAP2K enzyme MKK4 in the JNK signaling cascade. This 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, raising the possibility that JSP1 may offer a new perspective on the study of various disorders associated with aberrant JNK signaling. We have continued our characterization of mice that contain a targeted deletion of the *jsp1* gene, particularly with respect to the role of JSP1 as a positive regulator of JNK signaling in the neurodegenerative processes that lead to Parkinson's disease.

In addition to a single DSP catalytic domain, JSP1 contains a potential myristoylation site at its amino terminus (M*G*NG). Among the members of the PTP family, this is unique to JSP1 and its closest relative DUSP15/JSP2. Recent studies in the lab have focused on investigating the significance of this myristolyation site. In collaboration with Tom Neubert (Skirball Institute, New York University), we used mass spectrometry to confirm the presence of a myristoyl group at glycine 2 in the wild-type protein immunoprecipitated from cell lysates, but not in a JSP1 mutant in which the glycine was mutated to alanine. Using JSP1 tagged at the carboxyl terminus with green fluorescent protein (GFP), we observed that the wild-type enzyme colocalized with Golgi markers and is excluded from the nucleus, whereas the nonmyristoylated form of JSP1 is uniformly distributed throughout the cell. In addition, the nonmyristoylated form of the enzyme displays impaired ability to induce JNK activation and c-Jun phosphorylation. Wild-type JSP1 also has a shorter half-life than the nonmyristoylated form. Studying the impact of myristoylation on JSP1 function proved to be difficult because ectopic expression of the wild-type enzyme is poorly tolerated by cells. Expression of wild-type JSP1 led to a cell phenotype reminiscent of apoptotic cell death, which is less pronounced in cells expressing the myristoylation site mutant. Thus, these studies indicate an important role for myristoylation-dependent localization of JSP1 in the regulation of JNK signaling.

Dephosphorylation of the carboxy-terminal tyrosyl residue of the DNA-damage-related histone H2A.X is mediated by the protein phosphatase Eyes Absent. Double-stranded breaks (DSBs) are an extremely deleterious form of DNA damage with the potential for cell death and carcinogenesis. Therefore, tight control over the repair process is required. The reversible phosphorylation of histone H2A.X is an important component of the cellular response to DSBs, natural or imposed, in what is beginning to be described as an "epigenetic landscape" for DNA damage and repair. The variant histone H2A.X comprises ~10% of the H2A pool in mammalian cells and contains a unique carboxy-terminal segment with sites of regulatory phosphorylation. Within minutes of genotoxic stress, such as ionizing radiation, histone H2A.X becomes phosphorylated on Ser-139 in its carboxy-terminal segment by members of the PIKK family of Ser/Thr kinases, including ATM. Phosphorylated H2A.X decorates a broad region of ~1 Mb flanking the break, which serves as a platform for recruiting molecules involved in DNA-damage repair and signaling. These regions are referred to as γH2A.X foci. In mammalian cells, histone H2A.X also possesses a Tyr residue at its carboxyl terminus (Tyr-142), which has been shown by our collaborator David Alliss (The Rockefeller Uni-

versity) to be constitutively phosphorylated under normal growth conditions. Following DNA damage, such as in response to ionizing radiation, this residue becomes dephosphorylated, while γH2A.X, the Ser-139 phosphorylated form, appears. Phosphorylation of Tyr-142 is catalyzed by an atypical protein kinase WSTF, suppression of which by RNAi led to a decrease in Tyr phosphorylation of H2A.X and a rapid decline in the level of γH2A.X after ionizing radiation. Phosphorylation of Ser-139 and formation of γH2A.X foci were also reduced in cells expressing H2A.X in which Tyr-142 was mutated to non-phosphorylatable Phe or Leu residues, suggesting that the phosphorylation/dephosphorylation of these sites may be coordinated in a poorly understood "cross-talk" pathway. Clearly, the identity of the phosphatase(s) that dephosphorylates Tyr-142 is a critical component of this novel aspect of the DNA-damage response, because it would be part of the enzyme system responsible for governing the steady-state balance of this regulatory phosphorylation mark.

Eyes Absent (EYA), which is known to play a major role in tissue and organ development in many organisms, is the first example of a transcription factor with intrinsic phosphatase activity. It is a member of the haloacid dehalogenase (HAD) superfamily, which uses critical Asp residues at the active site to catalyze dephosphorylation. We determined the crystal structure of the catalytic domain of EYA2 and noted a striking feature: the clustering of acidic residues around the active site to present a negatively charged surface. This suggests that substrates of the enzyme may have basic properties. Other EYA family members share ~60% identity in this region of the protein, including 100% conservation of the surface acidic residues, consistent with them adopting a fold similar to that of EYA2. Considering the known functions of EYA in the nucleus and the fact that histones are recognized as highly basic proteins, we sought to determine whether EYA could dephosphorylate histone H2A.X as a substrate. Our data indicate that EYA3, and likely other EYA family members (EYA 1, 2, and 4), may function as a PTP that dephosphorylates Tyr-142 in histone H2A.X in vivo. We observed in assays in vitro that EYA demonstrated specificity for carboxy-terminal Tyr residue Tyr-142 in H2A.X (Fig. 1). Although there was a robust activity against the pTyr-142 H2A.X peptide, EYA2 and EYA3 did not dephosphorylate other pTyr peptides modeled on sequences that are known to engage signaling molecules. In contrast, PTP1B readily dephosphorylated these SH2 domain peptide ligands, but was inactive against the pTyr-142 H2A.X peptide. In the initial reports of characterization of the phosphatase activity of EYA, there was some disagreement about whether it displayed specificity for Tyr residues in proteins or could be viewed as a dual-specificity phosphatase. Our data provide further support for pTyr specificity of the EYA phosphatases. In contrast to the pTyr-142 peptide, EYA displayed ~10-fold lower activity toward the same peptide that was phosphorylated on the residue equivalent to Ser-139 of H2A.X. Furthermore, the presence of phosphate on the Ser residue was inhibitory to dephosphorylation by EYA (Fig. 1). This indicates not only a striking preference of EYA2 and EYA3 for the dephosphorylation of pTyr-142, but also raises the possibility that the dephosphorylation of the

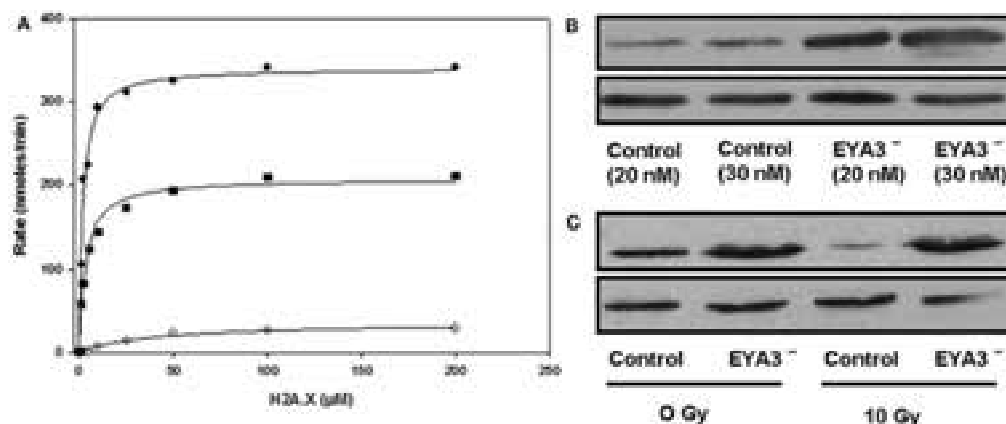

FIGURE 1 Identification of histone H2A.X as a physiological substrate of Eyes Absent (EYA). (*A*) Dephosphorylation of histone H2A.X-derived peptide substrates by EYA3. Shown are monophosphorylated H2A.X pTyr-142 (*closed circles*), monophosphorylated pSer-139 (*open circles*), and bisphosphorylated H2A.X pSer-139p/pTyr-142 (*closed squares*). Effects of suppression of eya3 by RNAi on the basal phosphorylation of Tyr-142 in histone H2A.X (*B*) and DNA-damage-induced dephosphorylation of H2A.X. (*C*) (*Upper panels*) Anti-H2A.X-pTyr-142 phospho-specific antibody; (*lower panels*) anti-H2A.X antibody.

carboxy-terminal Tyr residue may be influenced by phosphorylation of the neighboring residue Ser-139 in a "cross-talk" pathway. We noted that suppression of *eya3* by RNAi in U2OS cells led to increased basal phosphorylation and abrogated DNA-damage-induced dephosphorylation of Tyr-142 in histone H2A.X, consistent with a role for EYA3 as a physiological H2A.X phosphatase (Fig. 1). In addition, suppression of *eya3* was accompanied by a pronounced increase in cell death. Increased cell death has also been reported to accompany suppression of EYA family members in other systems. Most often, γ phosphorylation of H2A.X at Ser-139 is equated with "sensing" DSBs, leading to DNA repair in the context of a chromatin template. It is interesting to note, however, that histone H2A.X not only has been shown to function as a "caregiver" of the genome in DNA repair, but has also been implicated in the control of apoptosis in a poorly understood response. The importance of these observations is that not only do they provide the first indications of a physiological substrate of EYA, but they also suggest that the interaction of EYA and H2A.X may have a critical role in directing the choice that a cell makes regarding whether to undergo apoptosis or the process of DNA repair.

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HAPLOINSUFFICIENCY AND SENESCENCE IN CANCER

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PTEN is a tumor suppressor that is among the most frequently lost or mutated genes of human cancer. It is unique in directly opposing the enzymatic activity of phosphoinositol-3 kinases (PI3K) and the downstream proto-oncogene Akt kinase, which promotes cell survival and proliferation. This signaling pathway is deregulated in a majority of, for example, breast, prostate, and brain tumors. By modeling *Pten* loss in mice, we have discovered how cells in the prostate can sense the complete loss of *Pten* and respond to this insult by withdrawing into cellular senescence, which is now recognized as an early cell-intrinsic human antitumoral defense mechanism. However, we found that cells fail to respond similarly to more subtle deregulation of Pten protein as exemplified by its incomplete loss and that this leads to cancer by a process that we term "senescence bypass." This finding, combined with the senescence response, suggests that tumors should favor retention of some PTEN. Indeed, many human cancers present with incomplete *PTEN* loss, entirely consistent with these findings and establishing *PTEN* as a haploinsufficient tumor suppressor in such tissues.

Our studies of senescence bypass mechanisms have so far revealed two molecular scenarios. In one, tissues will spontaneously reduce Pten levels just enough to cross the threshold for abnormal proliferation. In the second scenario, partial inactivation of *Pten* and of a select cooperating tumor suppressor (e.g., the nuclear body-forming protein Pml or the cell cycle inhibitor p27) will suffice to produce senescence-free cancers that form only in this specific combination. Importantly, analysis of human tissues has confirmed the relevance of these two scenarios in prostate and colon cancer patients, to name a few. Thus, our genetic analysis of tumor progression in the mouse is allowing us to develop and test novel conceptual and mechanistic frameworks for cancer initiation and progression.

Nuclear versus Cytoplasmic Akt Activity in Cancer

M. Chen, D. Grace [in collaboration with A. Newton, University of San Diego, California]

Through our previous studies, we have learned that not only quantitative, but also qualitative, regulation of Akt kinase controls Pten mutant tumorigenesis and its progression via a senescence defeat or senescence bypass pathway. The qualitative change in active Akt kinase localization from the plasma membrane to the nucleus is a direct consequence of loss of promyelocytic leukemia (PML) nuclear bodies (NBs). We could show that in mice, Pml achieves pAkt inactivation through its ability to recruit both Akt and its phosphatase PP2a into these bodies, resulting in specifically nuclear Akt inactivation. These findings demonstrated the importance of coordinated Akt *inactivation* and revealed that this process is efficiently achieved through the phosphatase PP2a. Furthermore, it highlighted how deregulating cellular partitioning of Akt activity via control of phosphatases could bypass the senescence response.

Recently, a second direct phosphatase of Akt, named PHLPP, has been identified. PHLPP joins PTEN and PP2a phosphatases with the potential of regulating tumorigenesis. In contrast to the qualitative changes mentioned above, PHLPP controls the amplitude of Akt activation, similar to PTEN. However, it is unclear whether (1) PHLPP is a bona fide tumor suppressor and (2) in the context of *PTEN*-loss, loss of PHLPP bypasses senescence (similar to PP2a targeting) or triggers a senescence response in vivo (similar to complete PTEN loss). We have gathered strong evidence supporting a tumor-suppressive role for the *PHLPP* gene in human cancers of various tissues. To validate its role in vivo, we are crossing *Phlpp* null mice with *Pten* mutant mice. This analysis will allow us to define tissues where *Phlpp* is essential for tumor suppression and answer the question if and when a tumor wants to achieve maximal Akt pathway activation.

Nuclear PTEN and Cancer

J. Murn [in collaboration with B. Carver, W. Gerald, and C. Sawyers, Memorial Sloan-Kettering Cancer Center, New York]

Despite its plasma membrane function, PTEN has been consistently observed in cell nuclei, but the mechanism and relevance of this localization have remained unclear. We have recently resolved this paradox by demonstrating that contrary to polyubiquitination, nuclear PTEN import depends on its monoubiquitination and that mutation of the main PTEN ubiquitination site abolishes import in vitro and in patients, giving rise to inheritable Cowden's

disease because of low cytoplasmic PTEN stability. But most notably, this mutant retains catalytic activity, demonstrating that PTEN nuclear import is essential for tumor suppression. These findings exemplify an elemental insight into cancer biology by demonstrating how the collaboration of a genetic lesion (the inherited mutation) with a posttranslational cellular response (enhanced degradation) cooperates in tumorigenesis. Through this analysis, we have furthermore unraveled a link between two critical means of PTEN regulation, namely, stability and nucleocytoplasmic shuttling. We are currently investigating the mediators of both processes in vitro by using RNA interference (RNAi) approaches that can be expanded to in vivo analysis.

Regulation of PTEN Activity in Prostate Cancer Initiation and Treatment

H. Cho, J. Murn [in collaboration with C. Miething and P. Premsrirut, S. Lowe lab, Cold Spring Harbor Laboratory]

On the basis of our previously published work, the actual Pten protein levels inside prostatic epithelia decide between benign or malignant tumor formation (Trotman et al., *PLoS Biol. 3:* 385 [2003]), whereas complete *Pten* loss was shown to prevent tumorigenesis through the p53-dependent senescence response until p53 mutation paves the way for full-blown cancer. Accordingly, prostates of mice harboring partial *Pten* loss spontaneously degrade Pten to allow formation of prostate cancer. Yet, importantly, these lesions do not go on to completely lose Pten at the gene or protein level, a compelling illustration of their ability to maximize proliferation while avoiding the senescence response caused by complete *Pten* loss. Our analysis of human prostate cancer specimens also confirms frequent retention of clearly reduced PTEN levels. Therefore, by using mouse models with partial Pten loss, we found that spontaneous and subtle further reduction of Pten levels triggers activation of the downstream oncogenic kinase Akt, illustrating that a threshold must be crossed for initiation of cancer.

It is important to note that such spontaneous down-regulation of Pten is never observed in prostates of wild-type animals, sometimes observed in $Pten^{+/-}$ prostates (30% of mice), and is always occurring in $Pten^{hy/-}$ animals (mice with one-fourth of normal Pten levels; see Fig. 1). These observations strongly suggest that lower Pten levels increase the probability of its spontaneous reduction below the Akt-activating and tumor-promoting threshold. Thus, they demonstrate the importance of stable Pten levels in a tissue *even after Pten has received a genetic hit*. This finding should form the basis of a therapeutic approach, and thus we set out to test the therapeutic effect of Pten up-regulation in the prostate. An important open question behind this approach is that restoration of PTEN might not just antagonize but also revert tumor growth, analogous to the concept of oncogene addiction, even after cells have suffered spontaneous cooperating lesions.

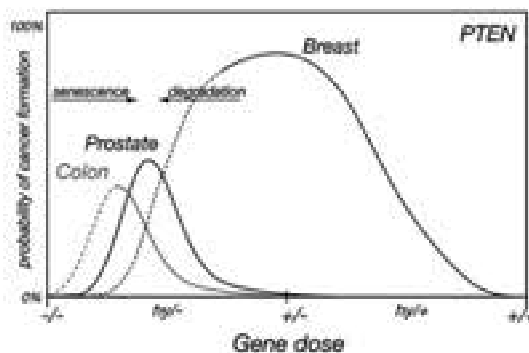

FIGURE 1 Pten haploinsufficiency and the senescence response.

To address this issue experimentally, we have designed reversibly inducible short-hairpin-based microRNAs in collaboration with the labs of Drs. Lowe and Hannon here at CSHL. As shown by Dr. Lowe's recent work, such hairpin designs can be used in vivo for reversible knockdown, which permits proof-of-principle studies on the therapeutic value of PI3K pathway control in specific genetic contexts. Through our approach, we have knocked down Pten levels in the mouse prostate until tumors have formed analogous to the models mentioned above. At this time, hairpin activity is blocked, Pten is restored, and tumor volume is monitored for regression using magnetic resonance imaging (MRI) methods. Of the 250,000 American men diagnosed with prostate cancer each year, about half will present with partial *PTEN* loss in their lesions, and thus, we expect our results to be of great importance in establishing the genetic framework for successful treatment of prostate cancer through PTEN stabilization or PI3K pathway inhibition.

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RAS AND RHO GTPASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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Ras and Rho family members have key roles in cellular activities controlling cell growth control, differentiation, and morphogenesis. Alterations that affect normal Ras and Rho function have been found to result in the development of several disease processes, including cancer and neurodevelopmental disorders. Our ultimate goal is to understand how aberrations in Ras and Rho signaling components contribute to the development of these disease/disorder processes. Toward this end, my lab has continued to define the functions of selected GTPases and their regulators and effectors in models of cancer and neurodevelopmental disorders. Below we highlight the main projects that have been carried out during the past year.

A CRITICAL ROLE FOR THE RHO-LINKED MENTAL RETARDATION PROTEIN OLIGOPHRENIN-1 IN GLUTAMATERGIC SYNAPSE MATURATION AND PLASTICITY

Mutations in genes encoding regulators and effectors of Rho GTPases have been found to underlie various forms of mental retardation (MR). *Oligophrenin-1* (*OPHN1*), which encodes a Rho-GTPase activating protein, was the first identified Rho-linked MR gene. It was initially identified by analysis of a balanced translocation t(X;12) observed in a female patient with mild MR. Subsequent studies have revealed the presence of *OPHN1* mutations in families with MR associated with cerebellar hypoplasia and lateral ventricle enlargement. All *OPHN1* mutations identified to date have been shown, or predicted, to result in OPHN1 loss of function. We previously demonstrated that the OPHN1 protein is highly expressed in the brain, where it is found in neurons of all major regions, including hippocampus and cortex, and it is present in both the axon and dendrites of principal neurons. Until now, however, the neuronal function of OPHN1 has been largely elusive. We had implicated OPHN1 in the regulation of spine morphology of CA1 hippocampal neurons, although it remained unclear whether OPHN1 controls spine formation or maintenance. Furthermore, the role of OPHN1 in the development or function of glutamatergic synapses remains to be elucidated.

By temporally and spatially manipulating *OPHN1* gene expression, we have now obtained evidence that postsynaptic OPHN1 has a key role in activity-dependent maturation and plasticity of excitatory synapses by controlling their structural and functional stability. Specifically, we find that synaptic activity through *N*-methyl-D-aspartate (NMDA) receptor activation drives OPHN1 into dendritic spines, where it forms a complex with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and selectively enhances AMPA-receptor-mediated synaptic transmission and spine size by stabilizing synaptic AMPA receptors. Consequently, decreased or defective OPHN1 signaling prevents glutamatergic synapse maturation and causes loss of synaptic structure, function, and plasticity. These results indicate that normal activity-driven glutamatergic synapse development is impaired by perturbation of OPHN1 function. Thus, our findings link genetic deficits in *OPHN1* to glutamatergic dysfunction and imply that defects in early circuitry development are an important contributory factor to this form of MR.

THE RAC ACTIVATOR DOCK7 REGULATES NEURONAL POLARITY AND MIGRATION

DOCK7 is a member of the evolutionarily conserved DOCK180-related protein superfamily, which we identified as a novel activator of Rac GTPases. We found that DOCK7 is highly expressed in major regions of the brain during early stages of development and, importantly, that the protein is asymmetrically distributed in unpolarized hippocampal neurons and becomes selectively expressed in the axon. We then obtained evidence that DOCK7 has a critical role in the early steps of axon formation in cultured hippocampal neurons. Knockdown of DOCK7 expression prevents axon formation, whereas overexpression induces the formation of multiple axons. We further demonstrated that DOCK7 and Rac activation leads to phosphorylation and inactivation of the microtubule destabilizing protein stathmin/Op18

in the nascent axon and that this event is important for axon development. Thus, our findings unveiled a novel pathway linking the Rac activator DOCK7 to a microtubule regulatory protein and they highlight the contribution of microtubule dynamics to axon development.

More recently, we set out to monitor neuronal polarization during the migration of newborn developing neurons within an intact brain structure and to establish DOCK7's role in this process. In particular, we implemented the in utero electroporation method that enables highly efficient introduction of vectors coexpressing proteins or short hairpin RNAs of interest with a fluorescent protein marker (e.g., EGFP or tdTomato) into ventricular zone (VZ) cells in embryonic cerebral cortices. Interestingly, we found that DOCK7 knockdown leads to inhibition of normal cortical migration. We are currently further determining the role of DOCK7 in the polarization and migration of cortical neurons in brain slices and are also assessing its involvement in the differentiation of living cerebellar granule cells. Together, these studies will contribute to a better understanding of the determinants/events involved in neuronal polarization, differentiation, and migration and the role of DOCK7 in these important processes.

ROLE OF RAP1 SIGNALING IN MORPHOGENETIC PROCESSES

The Rap1 protein, a member of the Ras family, was initially identified as an antagonist of oncogenic Ras proteins; however, more recent studies indicate that the function of Rap1 is largely Ras independent. Increasing evidence points to a critical role for Rap1 in the control of epithelial morphogenesis, and recent studies suggest a dysregulation of Rap1 signaling in malignant processes. How Rap1 proteins exert their effects in vivo has remained largely elusive. 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 perform functional analyses of their orthologs (Rap1 and Canoe) in the more genetically tractable *Drosophila* system. We found that both Rap1 and Canoe are required for epithelial migration events in the embryo and that Canoe acts as a downstream effector of Rap1 in these processes.

More recently, we identified a *Drosophila* Rap1-specific exchange factor, dPDZ-GEF, that is responsible for Rap1 activation in migrating embryonic epithelia. *Canoe* acts downstream from *Rap1* and *dPDZ-GEF* in this event. We demonstrated that dPDZ-GEF/Rap1/Canoe signaling modulates cell shape and apicolateral cell constriction in embryonic and wing disc epithelia. Interestingly, our data indicate that dPDZ-GEF signaling is linked to Myosin II function. Both *dPDZ-GEF* and *cno* show strong genetic interactions with the gene encoding Myosin II, and Myosin II distribution is severely perturbed in epithelia of both mutants. These findings provide a first insight into the molecular machinery targeted by Rap signaling to modulate cell shape in epithelial morphogenesis at different developmental stages.

ROLE OF DOK PROTEINS IN MITOGENIC AND ONCOGENIC SIGNALING

Dok-1 (also called $p62^{dok}$) was initially identified as a tyrosine-phosporylated 62-kD protein associated with Ras-GAP in Ph^{+} chronic myeloid leukemia (CML) blasts and in v-Abl-transformed B cells. This protein was termed Dok (*d*ownstream *o*f *k*inases), because it was also found to be a common substrate of many receptor and cytoplasmic tyrosine kinases. Subsequently, six additional Dok family members have been identified. Among them, Dok-1 and Dok-2 share the ability to bind to a negative regulator of Ras, Ras-GAP. We previously described that Dok-1 attenuates growth-factor-induced cell proliferation and that Dok-1 inactivation in mice causes a significant shortening of the latency of the fatal myeloproliferative disease induced by $p210^{bcr\text{-}abl}$. Strikingly, in collaboration with Dr. Pandolfi's group at Harvard Medical School, we also found that mice lacking both *Dok-1* and *Dok-2* spontaneously develop a CML-like myeloproliferative disease, likely resulting from increased cellular proliferation and reduced apoptosis. Thus, Dok proteins function as negative regulators of mitogenic and oncogenic signaling.

To further delineate the function of Dok-1 in oncogenic signaling, we examined the expression of Dok-1 protein in cells expressing various oncogene tyrosine kinases. Interestingly, we observed that $p210^{bcr\text{-}abl}$, v-Abl, and oncogenic forms of Src induce down-regulation of Dok-1 expression in a number of cell lines and that the reduction in the levels of Dok-1 protein requires tyrosine kinase activity of these oncogenes. Inactivation of tumor suppressors is an event that is observed in numerous tumors. Because the inactivation of the *Dok-1* gene accelerates the progression of CML in mouse models, whereas overexpression of *Dok-1* dramatically inhibits the proliferation of $p210^{bcr\text{-}abl}$-expressing cells as well as Src-induced transformation, our current findings suggest that oncogene-induced down-regulation of Dok-1 might be an event that contributes to the progression of tumorigenesis.

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NEUROSCIENCE

Neuroscientists at CSHL are striving to trace the remarkable process by which the developing brain gives rise to immensely complex neural networks, catalog and comprehend the full range of human genes implicated in mental illness, and demonstrate with great precision—in animals and humans—how genetic mutations and a host of related cellular anomalies give rise to disease-specific pathologies. These basic science investigations promise to spawn not only unprecedented diagnostic capabilities, but also, it is hoped, important new strategies for treating and perhaps even preventing some illnesses. They are certain to help unravel the still obscure mechanisms at work in devastating neurodevelopmental illnesses such as schizophrenia, depression, bipolar illness and other mood disorders, and autism and related "spectrum" disorders, as well as neurodegenerative illnesses such as Alzheimer's and Parkinson's diseases. The Swartz Center for Computational Neuroscience, headed by Anthony Zador, and the Stanley Center for Cognitive Genomics are among the coordinating centers for this vital research.

Holly Cline's lab is studying mechanisms in the brain controlling the growth of neurons, the generation of synapses, and the development of organized sensory projections between different brain regions. To understand the cellular events responsible for the stabilization of growing neuronal branches, the lab has delivered genes of interest into neurons and assessed the effects on synaptic transmission, specifically, in nerves that connect the retina and a part of the brain called the tectum involved in visual processing. Cline has learned that visual experience is a key modifier in how these circuits are built. This year, the lab demonstrated for the first time in living organisms (*Xenopus* tadpoles) that insulin receptor signaling in neurons regulates the maintenance of synapses, contributes to the processing of sensory information, and is involved in adjusting the plasticity of brain circuits in response to experience.

Because biological mechanisms of memory are highly conserved through evolution, many features of human memory are observed in simpler organisms such as fruit flies. Joshua Dubnau and colleagues identify genes that are important for memory and conserved across phyla. The hope is that many of these will be relevant to human memory. They recently discovered genes involved in controlling when and where specific proteins are synthesized within a neuron. These genes likely regulate neuronal communication during learning. The lab also seeks to discover how the neural circuitry of the fly brain works. They have recently shown that short- and long-term memories form in different sets of neurons: One circuit provides a memory that decays quickly and the other provides a memory that forms slowly but persists. Dubnau's team also explores how groups of genes interact to form memories, an approach designed to shed light on complex gene networks that likely underlie human cognitive disorders.

Grigori Enikolopov and colleagues study stem cells in the adult brain. They have generated several models to account for how stem cells give rise to progenitors and, ultimately, to neurons and are using these models to determine the targets of antidepressant therapies, to identify signaling pathways that control generation of new neurons, and to search for neuronal and neuroendocrine circuits involved in mood regulation. Recent experiments have suggested to the team a new model of how stem cells are regulated in the adult brain, with a focus on stem cells' "decision" on whether to divide—and embark on a path of differentiation—or remain quiescent. This model also explains why the number of new neurons decreases with advancing age. Recently, Enikolopov's group was part of a team that identified and validated the first biomarker that permits neuronal progenitor cells to be tracked, noninvasively, in the brains of living human subjects. The lab is now using this discovery to reveal how neurogenesis is related to the course of diseases such as depression, bipolar disorder, Alzheimer's, and Parkinson's.

Hiro Furukawa's lab is studying neurotransmission at the molecular level. They focus on two types of calcium ion channels: *N*-methyl-D-aspartate (NMDA) receptors and calcium homeostasis modulators (CALHM). Both are involved in the regulation of neuronal activities and in the pathogenesis of Alzheimer's disease. NMDA receptors are very large molecules whose three-dimensional atomic structure Furukawa's group has undertaken to solve by dividing them into several domains. They seek to understand the pharmacological specificity of ligand and modulator bindings in different subtypes of NMDA receptors in order to provide a blueprint for future

drug design. A mutation of the gene coding for CALHM has been implicated in late-onset Alzheimer's disease. The team is working to reveal the mechanism by which this calcium-specific ion channel opens by uncovering the molecule's architecture. Such structural information promises to be useful in the design of novel drugs for treatment of Alzheimer's.

Adam Kepecs studies the neurobiological principles by which the brain makes decisions. He and his colleagues view decisions as elementary units of behavior, from which more complex behaviors are assembled. Yet even simple decisions involve the integration of sensory and memory information with emotional and motivational attributes, requiring the concerted action of millions of neurons across brain regions. Therefore, they take an integrative approach, combining experiments involving well-controlled rodent behavior with electrophysiology, molecular perturbations, and quantitative analysis. Their current work seeks to elucidate the neurocomputational principles of decision-making, attempting to capture more elusive attributes such as emotion, motivation, or confidence. This year, Kepecs, in a collaboration with Zachary Mainen and others here at CSHL, discovered neural signals for confidence in the rat prefrontal cortex. Their study suggests that confidence estimation is a fundamental information-processing mechanism in the brain, shared widely across species, and not strictly confined to those, such as humans, who are self-aware.

The mammalian brain is a miracle of self-assembly, a process that begins prenatally and continues well into the postnatal period. Alexei Koulakov and colleagues are trying to determine the mathematical rules by which the brain assembles itself, with particular focus on the formation of sensory circuits such as those involved in visual perception and olfaction. The visual system of the mouse was chosen for study in part because its components, in neuroanatomical terms, are well understood. What is not known is how projections are generated that lead from the eye through the thalamus and into the visual cortex, how an individual's experience influences the configuration of the network, and what parameters for the process are set by genetic factors. Even less is known about the assembly of the neural net within the mouse olfactory system, which, in the end, enables the individual to distinguish one smell from another with astonishing specificity and to remember such distinctions over time. These are among the challenges that engage Koulakov and his team.

Dysfunction of excitatory, glutamatergic synapses in the brain is believed to have an important role in the pathogenesis of major psychiatric disorders, including schizophrenia and depression. But what are the causes? Where in the brain does this dysfunction occur? How does it result in the behavioral symptoms of illness? To address these issues, Bo Li and colleagues are studying normal synaptic plasticity and disease-related synaptic changes in brain circuits involved in schizophrenia and depression. Their long-term goal is to develop methods allowing the manipulation of activity in specific brain circuits in order to change disease-related behaviors. They will use a variety of methodologies, including patch-clamp recording and calcium imaging of labeled neurons, two-photon imaging of spine morphology and tagged receptors, in vivo virus injection, RNAi-based gene silencing, activation of specific axon terminals using light-gated cation channels, activation or silencing of specific brain regions using transgenes, and assessment of the behavioral consequences of certain manipulations.

To better understand neuronal circuits, Josh Huang and colleagues have developed novel means of visualizing the structure and connectivity of different cell types at high resolution in living animals and of manipulating the function of specific cell types with remarkable precision. Huang is particularly interested in circuits that use GABA, the brain's primary inhibitory neurotransmitter. The lab's work has direct implications in neurological and psychiatric illness such as autism and schizophrenia, which involve altered development and function of GABAergic circuits. This past year, they discovered that neurons connect to very specific partners at very specific spots in the developing cerebellum, thanks to an underlying framework of molecular "guides" called glial cells that nudge nerve fibers to grow in the right direction and make the right contacts. The lab also made good progress in studying perturbations in the developing GABAergic system in a mouse model of Rett's syndrome, one of the autism spectrum disorders.

Zachary Mainen studies the neural basis of mammalian behavior and cognition. He focuses on understanding the nature of the electrical activity of single neurons, the "currency" with which genes and molecules express themselves in the functioning brain. His lab also studies neural coding, or how information is represented in characteristic "spikes" of neuronal electrical discharges. This year, in collaboration with Adam Kepecs and others here at CSHL, he published results of studies in rats suggesting that the estimation of confidence that underlies decision-making may be the product of a very basic kind of information processing in the brain, shared widely across species. Separately, Mainen showed that the superior colliculus, a structure implicated in visu-

ally guided eye movements, has a much more general role in spatial decision-making than previously thought.

Pavel Osten's lab is developing several approaches to uncover pathologies responsible for mental disorders, including autism spectrum disorders (ASDs) and schizophrenia, and neurodegenerative illnesses such as Parkinson's and Alzheimer's. In neuropsychiatric disorders, Osten is exploring the link between candidate genes and common "core" dysfunctions at the level of neural circuitry, specifically in mouse models of schizophrenia and autism. The ability to systematically and rapidly compare brain functions, analyze which brain regions are affected, and determine which cellular networks within these regions are altered is vital in the larger effort to understand how genes perturb biology, giving rise to symptomatic behaviors. Osten and collaborators are also creating automated systems that will take a sample of brain tissue as input, generate a three-dimensional representation of its neural circuit activity, and then map it to a brain atlas. The object is to reveal how neural circuits are wired during development, to make possible precise characterization of neural circuit disruptions in mouse models.

When we learn an association, information from two different sensory streams is somehow bound together in the brain. For example, the smell of spoiled milk vividly evokes the taste of an injudicious gulp. How are odor and taste information represented in terms of neural spike trains, and how does learning modify those representations to form associative links among stimuli? These are among the questions that drive research in Glenn Turner's lab. His team addresses these questions by using a combination of electrophysiological, genetic, and computational approaches in the fruit fly. They directly monitor activity of neurons in the brain of an intact fly with whole-cell patch-clamp recordings. Using molecular genetics techniques, they are able to manipulate neural activity to directly test their predictions about neural coding, at the level of both spike trains and behavior. Currently, they focus on representations of smell and taste.

Anthony Zador and colleagues study how brain biology gives rise to higher-level properties such as complex behavior. They have focused on how the cortex processes sound, how that processing is modulated by attention, and how it is altered in pathology. In the lab's "core assay," the response of single neurons to sound stimuli is examined under distinct behavioral conditions. In animal models of autism, the team is trying to link an inability to screen out background sounds with changes in neural circuits. Separately, by showing that a very small minority of available auditory neurons in a rat cortex react strongly when exposed to a specific sound, the lab has challenged the standard model of sound representation. This year, Zador's team generated evidence supportive of an alternative theory of information processing in the brain, showing that animals in the midst of decision-making have the ability to distinguish incoming signal spikes separated by as little as 3 milliseconds. This lends credence to a timing model of information processing as opposed to one based on the rate of signal firing.

Yi Zhong's lab studies the neural basis of learning and memory. They work with fruit fly models to study genes involved in human cognitive disorders, including neurofibromatosis, Noonan's syndrome, and Alzheimer's disease. Mutations of the neurofibromatosis 1 *(NF1)* gene cause learning defects and neurofibromas—nerve-sheath tumors that split apart nerve fibers. The lab's analyses of *Drosophila NF1* mutants have revealed how expression of the mutant gene affects a pathway crucial for learning. They have also discovered that the *NF1* gene and a gene called *corkscrew*, both implicated in Noonan's, share a biochemical pathway. This year, they linked specific genetic defects in Noonan's with long-term memory deficiencies that are among the symptoms of the illness. They postulate a "spacing effect," which, if addressed with remedial learning methods, might help to address the impairment. Zhong also contributed to a study in which plaques implicated in Alzheimer's memory loss were experimentally reduced in the fruit fly brain by overexpressing a human gene that codes for the production of an enzyme called neprilysin, or NEP.

How does the brain encode stimuli from the outside world to generate specific perceptions that, in turn, trigger complex behaviors? How is the brain shaped by sensory experience and what modifications occur in neuronal circuits that allow us to learn and remember? These are questions guiding the work of CSHL Fellow Florin Albeanu, who is using the olfactory bulb in living mice as the subject of his current studies. Rodents depend on olfaction for finding food and mates and avoiding predators. Airborne chemicals, translated into neuronal signals by specific receptors in the nose, are sent directly to the olfactory bulb. Although readily accessible, little is known about the neuronal processing that occurs there. Technological advances in optical imaging enable Albeanu to monitor patterns of activity at unprecedented synaptic resolution, in real time, as animals "behave."

BRAIN DEVELOPMENT AND PLASTICITY

H. Cline J. Bestman, K. Bronson, K. Burgos, S.-L. Chiu, J. Demas, M. Hiramoto, S.-Y. Lee, J. Lee-Osbourne, J. Li, P. Sharma, W. Shen, V. Thirumalai

Our research explores mechanisms regulating brain circuit development. My lab addresses this fundamental question by examining the development of the visual system in *Xenopus* tadpoles and the development of the spinal cord in zebra fish. We focus on activity-dependent regulation of circuit function. These studies have relevance to fundamental features of circuit development.

Rapid Activity-dependent Delivery of the Neurotrophic Protein CPG15 to the Axon Surface of Neurons in Intact *Xenopus* Tadpoles

I. Cantallops, H. Clline

CPG15 (also known as Neuritin) is an activity-induced GPI-anchored axonal protein that promotes dendritic and axonal growth and accelerates synaptic maturation in vivo. Here, we show that CPG15 is distributed inside axons and on the axon surface. CPG15 is trafficked to and from the axonal surface by membrane depolarization. To assess CPG15 trafficking in vivo, we expressed an ecliptic pHluorin (EP)-CPG15 fusion protein in optic tectal explants and in retinal ganglion cells of intact *Xenopus* tadpoles. Depolarization by KCl increased EP-CPG15 fluorescence on axons. Intraocular kainic acid (KA) injection rapidly increased cell surface EP-CPG15 in retinotectal axons, but coinjection of tetrodotoxin (TTX) and KA did not. Consistent with this, we find that intracellular CPG15 is localized to vesicles and endosomes in presynaptic terminals and colocalizes with synaptic vesicle proteins. The results indicate that the delivery of the neurotrophic protein CPG15 to the axon surface can be regulated on a rapid timescale by activity-dependent mechanisms in vivo.

Homer Proteins Shape *Xenopus* Optic Tectal Cell Dendritic Arbor

K. Van Keuren-Jensen, H. Cline

Considerable evidence suggests that the Homer family of scaffolding proteins contributes to synaptic organization and function. We investigated the role of both Homer 1b, the constitutively expressed and developmentally regulated form of Homer, and Homer 1a, the activity-induced immediate-early gene, in dendritic arbor elaboration and synaptic function of developing *Xenopus* optic tectal neurons. We expressed exogenous Homer 1a or Homer 1b in developing *Xenopus* tectal neurons. By collecting in vivo time-lapse images of individual, enhanced green fluorescent protein (EGFP)-labeled and Homer-expressing neurons over 3 days, we found that Homer 1b leads to a significant decrease in dendritic arbor growth rate and arbor size. Synaptic transmission was also altered in developing neurons transfected with Homer 1b. Cells expressing exogenous Homer 1b over 3 days had significantly greater AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid) to NMDA (*N*-methyl-D-aspartate) ratios, and increased AMPA miniature excitatory postsynaptic currents (mEPSC) frequency. These data suggest that increasing Homer 1b expression increases excitatory synaptic inputs, increases synaptic maturation, and slows dendritic arbor growth rate. Exogenous Homer 1a expression increases AMPA mEPSC frequency, but it did not significantly affect tectal cell dendritic arbor development. Changes in the ratio of Homer 1a to Homer 1b may signal the neuron that overall activity levels in the cell have changed, and this in turn could affect protein interactions at the synapse, synaptic transmission, and structural development of the dendritic arbor.

Endogenous Dopamine Suppresses Initiation of Swimming in Pre-feeding Zebra Fish Larvae

V. Thirumalai

Dopamine is a key neuromodulator of locomotory circuits, yet the role that dopamine has during development of these circuits is less well understood. We found that dopamine depresses the activity of swim circuits in larval zebra fish. Zebra fish larvae exhibit marked changes in swimming behavior between 3 days postfertilization (dpf) and 5 dpf. We found that swim episodes were fewer and of longer durations at 3 dpf than at 5 dpf. At 3 dpf, application of dopamine as well as bupropion, a dopamine reuptake blocker, abolished spontaneous fictive swim episodes. Blocking D2 receptors increased frequency of occurrence of episodes, and activation of adenylyl cyclase, a downstream target inhibited by D2 receptor signaling,

blocked the inhibitory effect of dopamine. Dopamine had no effect on motor neuron firing properties, input impedance, resting membrane potential, or the amplitude of spike after hyperpolarization. Application of dopamine either to the isolated spinal cord or locally within the cord does not decrease episode frequency, whereas dopamine application to the brain silences episodes, suggesting a supraspinal locus of dopaminergic action. Treating larvae with 10 μM MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) reduced catecholaminergic innervation in the brain and increased episode frequency. These data indicate that dopamine inhibits the initiation of fictive swimming episodes at 3 dpf. We found that at 5 dpf, exogenously applied dopamine inhibits swim episodes, yet the dopamine reuptake blocker or the D2 receptor antagonist have no effect on episode frequency. These results led us to propose that endogenous dopamine release transiently suppresses swim circuits in developing zebra fish.

The RNA-binding Protein CPEB Controls Dendrite Growth and Neural Circuit Assembly In Vivo

J. Bestman

Visual system development requires experience-dependent mechanisms that regulate neuronal structure and function, including dendritic arbor growth, synapse formation, and stabilization. Although RNA-binding proteins have been shown to affect some forms of synaptic plasticity in adult animals, their role in the development of neuronal structure and functional circuitry is not clear. Using two-photon time-lapse in vivo imaging and electrophysiology combined with morpholino-mediated knockdown and expression of functional deletion mutants, we demonstrate that the mRNA-binding protein cytoplasmic polyadenylation element-binding protein1 (CPEB1) affects experience-dependent neuronal development and circuit formation in the visual system of *Xenopus laevis*. These data indicate that sensory experience controls circuit development by regulating translational activity of mRNAs.

Insulin Receptor Signaling Regulates Synapse Number, Dendritic Plasticity, and Circuit Function In Vivo

S.-L. Chiu, C.-M. Chen

Insulin receptor signaling has been postulated to have a role in synaptic plasticity; however, the function of the insulin receptor in the central nervous system (CNS) is not clear. To test whether insulin receptor signaling affects visual system function, we recorded light-evoked responses in optic tectal neurons in living *Xenopus* tadpoles. Tectal neurons transfected with dominant-negative insulin receptor (dnIR), which reduces insulin receptor phosphorylation, or morpholino against insulin receptor, which reduces total insulin receptor protein level, have significantly smaller light-evoked responses than controls. dnIR-expressing neurons have reduced synapse density as assessed by electron microscopy, decreased AMPA mEPSC frequency, and altered experience-dependent dendritic arbor structural plasticity, although synaptic vesicle release probability, assessed by paired-pulse responses and synapse maturation, assessed by AMPA/NMDA ratio and ultrastructural criteria, are unaffected by dnIR expression. These data indicate that insulin receptor signaling regulates circuit function and plasticity by controlling synapse density.

The Regulation of Dendritic Arbor Development and Plasticity by Glutamatergic Synaptic Input: A Review of the Synaptotrophic Hypothesis'

H. Cline [in collaboration with K. Haas, University of British Columbia, Canada]

The synaptotropic hypothesis, which states that synaptic inputs control the elaboration of dendritic (and axonal) arbors, was articulated by Vaughn in 1989. Today, the role of synaptic inputs in controlling neuronal structural development remains an area of intense research activity. Several recent studies have applied modern molecular genetic, imaging, and electrophysiological methods to this question and now provide strong evidence that maturation of excitatory synaptic inputs is required for the development of neuronal structure in the intact brain. Nevertheless, some studies fail to show evidence that afferent inputs affect structural development. We review data that both support and refute the hypothesis with the expectation that understanding the circumstances when the data do and do not support the hypothesis will be most valuable.

NR2A and NR2B Have Distinct but Overlapping Roles in Shaping the Development of Dendritic Arbor Morphology In Vivo

R.C. Ewald, K.R. Van Keuren-Jensen, C.D. Aizenman

NMDA receptors (NMDARs) are important for neuronal development and circuit formation. The NMDAR subunits NR2A and NR2B are biophysically distinct and differentially expressed during development, but their individual contribution to structural plasticity is unknown. Here, we

test whether NR2A and NR2B subunits have specific functions in the morphological development of tectal neurons in living *Xenopus* tadpoles. Exogenous subunit expression and endogenous subunit knockdown shift the synaptic NMDAR composition toward NR2A or NR2B, as shown electrophysiologically. We analyzed the dendritic arbor structure and found evidence for both overlapping and distinct functions of NR2A and NR2B in dendritic development. Control neurons obtain regions of high local branch density in their dendritic arbor, whereas exogenous expression of either NR2A or NR2B decreases local branch clusters, indicating a requirement for both subunits in dendritic arbor development. Knockdown of endogenous NR2A, however, reduces local branch clusters compared to knockdown of NR2B, suggesting more complex NR2 signaling. Analysis of the underlying branch dynamics shows that exogenous NR2B-expressing neurons are more dynamic than control or exogenous NR2A-expressing neurons, demonstrating subunit-specific regulation of branch dynamics. However, visual experience-dependent increases in dendritic arbor growth rate seen in control neurons are blocked in both exogenous NR2A- and NR2B-expressing neurons. Furthermore, AMPAR-mediated synaptic transmission is decreased in neurons with knocked down or exogenous NMDAR composition, indicating overlapping roles for NR2A and NR2B in regulating synaptic transmission. Thus, we show that NR2A and NR2B have subunit-specific properties in dendritic arbor development but also overlapping functions, indicating a requirement for both subunits in neuronal development.

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GENETICS OF MEMORY IN *DROSOPHILA*

J. Dubnau A. Blum W. Li
M. Cressy H. Qin

Understanding complex behaviors such as memory will require a multidisciplinary approach that will include discovery and manipulation of the relevant genetic/cell signaling pathways and the relevant neural circuits. Work in genetic model systems such as *Drosophila* can contribute to our understanding in several ways. First, by enabling discovery of genes and genetic pathways underlying behavior, such model systems provide entry points for dissection of cellular mechanisms that are often conserved. Second, systematic manipulation of gene function in relevant anatomical loci of the brain allows a conceptual integration of findings from cellular and behavioral neuroscience. Our gene discovery efforts already have identified a role in memory for highly conserved pathway(s) involved with subcellular control of translational regulation. Many of these molecules have counterparts in vertebrates that also appear to have important roles in brain function and, in some cases, may be linked to human cognitive dysfunction. Our genetic investigations also provide insight to investigate the neural circuitry relevant to memory because gene expression patterns often suggest hypotheses that can be tested spatially to restricted genetic manipulations. Our recent findings demonstrate that short- and long-term memory are supported by anatomically distinct memory traces. This too appears to reflect a fundamental (but poorly understood) feature of memory formation. Finally, at the behavioral level, we are uncovering properties of learned fear that may be relevant to fear disorders in humans.

Synaptic Targets of Pumilio

J. Dubnau, W. Li

Using a series of genome-wide screens, we identified a role in memory for a number of mRNA-binding proteins (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). These include several with known roles in mRNA localization and translational control. In humans, cellular mechanisms of local translation are of relevance to the etiology of fragile-X mental retardation syndrome. Our genetic studies in *Drosophila* already support a role in memory for several components of this cellular machinery. These include *staufen* and *oskar*, which are known components of cellular mRNA localization machinery in oocytes, and *pumilio*, which is a translational repressor protein whose vertebrate orthologs are highly conserved but largely unstudied. Although *pumilio* function has been most carefully studied in the context of embryonic patterning, several recent findings demonstrate that *pumilio* also has an important role in the nervous system, including long-term memory formation (see, e.g., Dubnau et al., *Curr. Biol. 13:* 286 [2003]; Berger et al. 2008; Chen et al. 2008). In neurons, *pumilio* appears to have a role in homeostatic control of excitability via down-regulation of *para*, a voltage-gated sodium channel, and it may more generally modulate local protein synthesis in neurons via translational repression of *eIF-4E*. Aside from these, the biologically relevant targets of *pumilio* in the nervous system were largely unknown in any species.

We hypothesized that *pumilio* might have a role in regulating the local translation underlying synapse-specific modifications during memory formation. To identify relevant translational targets, we used an informatics approach (in collaboration with M. Zhang's lab here at CSHL) to predict *pumilio* targets among mRNAs whose products have synaptic localization (Chen et al. 2008). We then used both in vitro binding and two in vivo assays to functionally confirm the fidelity of this informatics screening method. We found that *pumilio* strongly and specifically binds to RNA sequences in the 3′UTR (untranslated region) of four of the predicted target genes, demonstrating the validity of our method. We then demonstrated that one of these predicted target sequences, in the 3′UTR of *discs large* (*dlg1*), the *Drosophila* PSD95 ortholog, can functionally substitute for a canonical Nanos response element (NRE) in vivo in a heterologous functional assay in the embryo. We also demonstrated that the endogenous *dlg1* mRNA can be regulated by *pumilio* in a neuronal context, the adult mushroom bodies (MBs), which is an anatomical site of memory storage (Fig. 1). Our current efforts focus on several additional neuronal targets of *pumilio*, construction of in vivo sensors to image local neuronal translation, and higher-throughput in vivo approaches to fully characterize the combinatorial action of *pumilio* and other regulators to control neuronal translation.

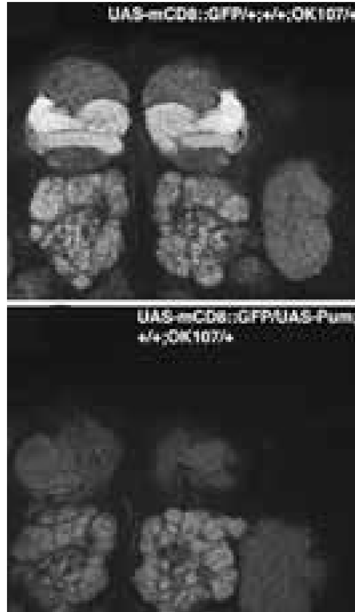

FIGURE 1 Dlg is repressed by overexpression of Pum in MB Kenyon cells. An MB-expressing Gal4 line (OK107) was used to drive expression of both UAS-mCD8::GFP and UAS-Pum transgenes. Shown are optical sections of the MB lobes. In wild type (*top*), Dlg expression is detected both in MB lobes and AL and in the MB peduncle (not shown). In contrast, Dlg expression is dramatically reduced in the MB lobes (*bottom*) and peduncle (not shown) of Pum overexpressing MBs. AL glomeruli, also stained by Dlg antibody, serve as an internal control.

Neural Circuitry of Memory

A. Blum, J. Dubnau

A common feature of memory and its underlying synaptic plasticity is that each can be dissected into short-lived forms involving modification or trafficking of existing proteins and long-term forms that require new gene expression. An underlying assumption of this cellular view of memory consolidation is that these different mechanisms occur within a single neuron. At the neuroanatomical level, however, different temporal stages of memory can engage distinct neural circuits, a notion that has not been conceptually integrated with the cellular view. We have investigated this issue in the context of aversive Pavlovian olfactory memory in *Drosophila*. Previous studies have demonstrated a central role for cAMP signaling in the MB. The Ca^{2+} responsive adenylyl cyclase *rutabaga* is believed to be a coincidence detector in one of the three principal classes of MB Kenyon cells. We have conducted the first systematic investigation of the requirements for cAMP signaling in each of the three major MB neuron subtypes for each of the temporal phases of memory (Blum and Dubnau, in press). We spatially restricted expression of the *rutabaga*$^{+}$ cDNA to each of the major subtypes of MB neurons and examined memory during a time course after training. Using this approach, we are able to separately restore short- and long-term memory traces to a *rutabaga* mutant with expression of *rutabaga* in different subsets of MB neurons (see, e.g., Fig. 2). Our findings suggest a model in which the learning experience initiates two parallel associations: a short-lived trace in MB γ neurons and a long-lived trace in α/β neurons (Blum and Dubnau, in press).

Enhancer/Suppressor Screening by Selective Breeding: Modeling Rubenstein-Taybi Syndrome in *Drosophila*

M. Cressy, J. Dubnau [in collaboration with P. Mitra and D. Valente, Cold Spring Harbor Laboratory]

Most quantitative phenotypes have a complex genetic etiology. Even in cases with an apparently “simple” Mendelian inheritance, epistatic interactions within genetic background can have profound impacts on phenotype. In addition to its obvious importance to our understanding of genotype to phenotype relationships, “sensitivity to genetic background” can impact both severity and clinical outcome of genetic disorders. Rubinstein-Taybi syndrome (RTS), for instance, is a rare congenital disorder characterized in part by mental retardation. In greater than 50% of patients, RTS is caused by mutations in one copy of the cAMP response element binding (CREB)-binding protein (CBP) located in 16p13.3. Mouse models of this disorder demonstrate that the cognitive defects of the CBP mutants can be rescued by pharmacological stimulation of cAMP signaling. Even with this relatively simple inheritance pattern, however, there is considerable clinical variability both among RTS patients and between CBP mutant mice in different genetic backgrounds.

Despite its widespread relevance, the mechanisms by which multigene interactions modulate phenotype are ill understood. Historically, experimental dissection of gene interaction has depended on two strategies: forward mutagenesis and selective breeding. Each of these approaches has its own limitations. Forward mutagenesis has traditionally been the workhorse for the genetic dissection of cellular mechanisms and entails a two-tiered strategy. First is the identification of individual genes that influence a particular phenotype, followed by analysis of pair-

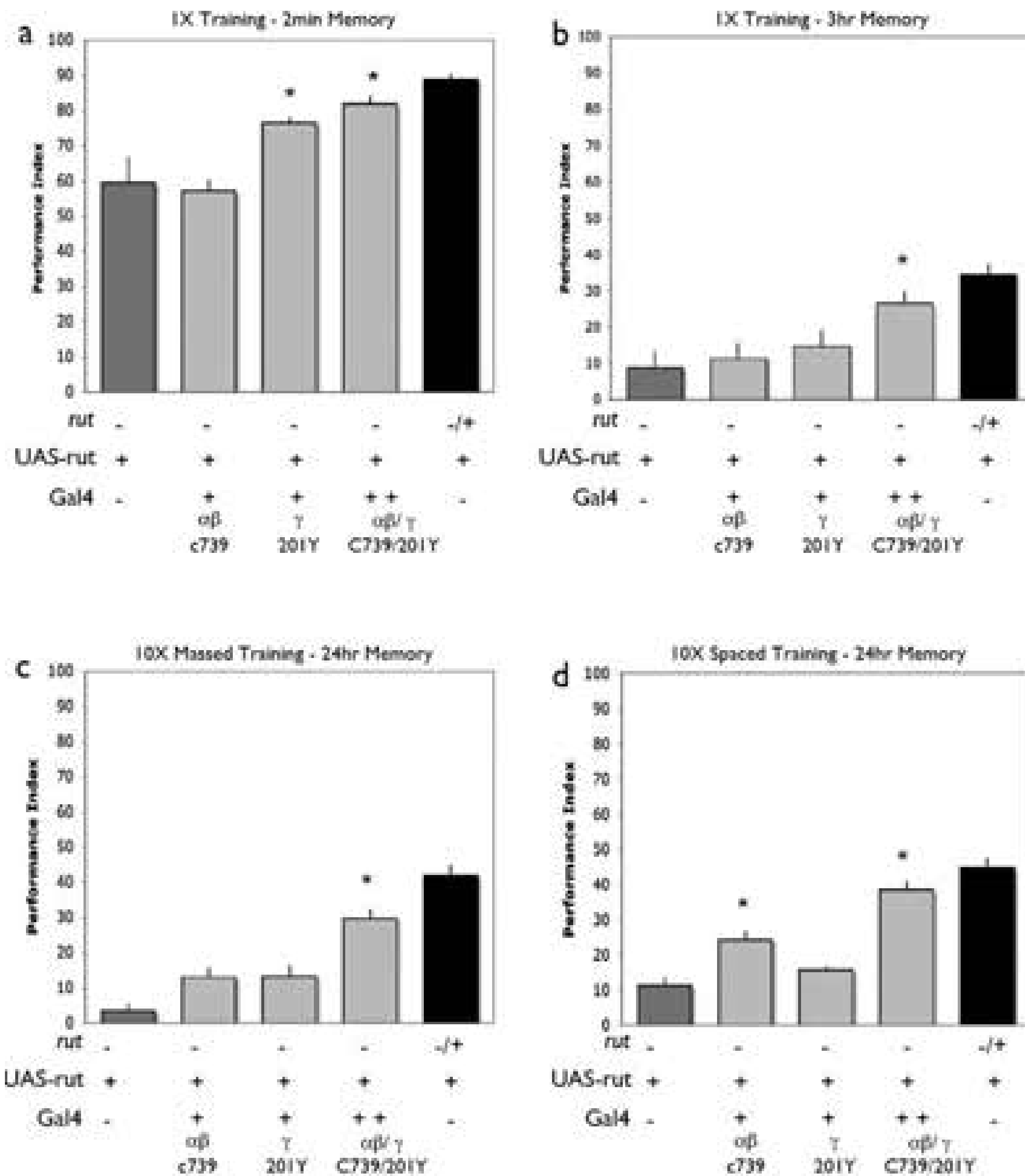

FIGURE 2 *rutabaga* expression in γ neurons supports short-term but not long-term memory. In contrast, *rutabaga* expression in α/β neurons supports long-term but not short term memory. Memory retention was tested 2 min (*a*) and 3 h (*b*) after a single training session as well as 24 hours after either massed (*c*) or spaced (*d*) training. Only spaced training gives CREB-dependent long-term memory. In each case, performance was compared among the following groups: rut2080 mutant males with a UAS-rut+ transgene but no Gal4 driver (rut2080/Y;UAS-rut), rut2080 heterozygous females with a UAS-rut+ transgene but no Gal4 driver (rut2080/+;UAS-rut), rut2080 mutant males with a UAS-rut+ transgene and either the 201Y or C739 drivers alone, or the 201Y and C739 Gal4 drivers combined, and rut2080 heterozygous females with a UAS-rut+ transgene and these Gal4 lines. In contrast with the rut2080/Y;UAS-rut mutant males, rut2080 mutant males with both a UAS-rut+ transgene and either the 201Y or 201Y combined with C739 Gal4 drivers exhibit nearly normal levels of performance measured 2 min after training, whereas C739 expression caused no improvement ($P < 0.05$, $N = 6$ for all groups) (*a*) and only expression combined with both the 201Y and C739 drivers significantly improved performance 3 h after a single training session ($P < 0.05$, $N = 8$ for all groups) (*b*). Only expression combined with both the 201Y and C739 drivers significantly improved performance 24 h after massed training ($P < 0.05$, $N = 18$ for all groups) (*c*). For 24 h after spaced training, expression with the C739 driver alone resulted in significant improvement of performance; however, this effect was augmented by combining both C739 and 201Y expression ($P < 0.05$, $N = 23$ for all groups). In all cases, no significant improvements were observed in control females that were rut2080/+;UAS-rut and contained a Gal4 line with the exception of flies carrying both the 201Y and C739 drivers combined after spaced training.

wise gene interactions via modifier screens. Although this strategy has been tremendously successful at revealing fundamental molecular mechanisms, it has not fully modeled the complexity of gene networks underlying phenotype. On the other hand, multigenerational selective breeding has been successfully used to study the genetic architecture underlying complex phenotypes across a wide variety of organisms. This strategy more closely models the multigene interactions that influence phenotype and that may be relevant to the natural evolution of phenotypic change. Unlike forward mutagenesis, however, when used on its own, this strategy does not elucidate the underlying molecular mechanisms.

We have developed a novel approach that combines the strengths of these two methods. Our approach relies on artificial selection over the course of multiple generations to "evolve" combinations of known and molecularly tagged gene variants that interact to modify the memory defect of the *rutabaga* adenylyl cyclase. This is an attempt to model the effects of genetic background on phenotypic severity in RTS and is also designed to use experimental evolution to mimic classical suppressor screens. We first established large populations of animals that are homozygous for a null mutation in the *rutabaga* gene. These populations were engineered to contain controlled heterogeneity at 23 loci that were first identified via a forward mutagenesis (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). Each animal in the starting population was homozygous for a different combination of three or four of these 23 mutations. Thus, the possible allele space is combinatorially quite large. The second stage of this project is multigenerational selective breeding to evolve combinations of alleles that are capable of suppressing the *rutabaga* learning defect. At this date, we have carried the selection on three replicate populations for 34 generations (Fig. 3). Control populations are allowed to drift in parallel. A robust response to selection is seen beginning at about generation 13. The third stage of this experiment involves a high-throughput genotyping to

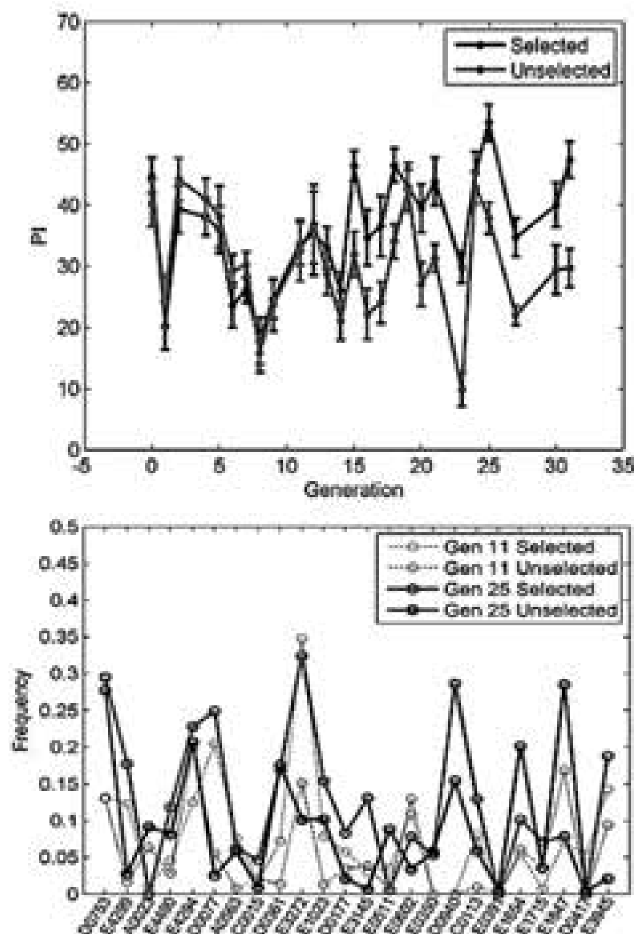

FIGURE 3 Experimental evolution of *rutabaga* suppression: Modeling gene interactions underlying the Rubenstein-Taybi syndrome. (*Top*) Selective breeding for combinations among 23 alleles that lead to improved learning in *rutabaga* mutants. Mean data are shown over 31 generations for three replicate selected populations (Morgan, Muller, Lewis) and three unselected populations (Bridges, Sturtevant, Dobzhansky). All animals are homozygous null for *rutabaga* and contain controlled heterogeneity at 23 loci that were first identified in a forward mutagenesis screen (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). The performance index (PI) for each population is determined at each generation using our standard Pavlovian olfactory learning assay. (*Bottom*) High-throughput multiplex polymerase chain reaction (PCR) was used to determine the genotypes of ~300 animals from each population. This genotyping was performed at generation 11 and 25 for each population. Allele frequencies for each of the 23 loci are shown. Six alleles with frequencies that are significantly elevated in the selected strains (E4299, D0077, E3272, E3145, D0940, and E3945) were chosen for mechanistic studies of pairwise and triplet interactions.

determine allele frequencies and coinheritance patterns across the replicate populations (Fig. 3). These data identify at least six alleles whose frequencies are significantly increased in the selected versus unselected populations. We now are testing the phenotypic consequences of each single, double, and triple mutant combination of these six alleles.

Extinction Learning: A Model for Posttraumatic Stress Disorder

H. Qin, J. Dubnau

Repeated representation of the conditioned stimulus (CS) without reinforcement of the unconditioned stimulus (US) results in the diminishment of conditioned responding. This process is called extinction (of Pavlovian conditioning). Extinction was first studied by Pavlov in 1927 and has been reported in both appetitive and aversive paradigms. Extinction is not simply erasing the original CS-US association but appears to be a new learning process that antagonizes the original memory. Although this type of learning is important biologically, it has also received attention because of its potential relevance to posttraumatic stress disorder (PTSD), in which an individual continues to fear a particular set of stimuli or a context. This debilitating fear can be highly resistant to extinction.

We are taking advantage of the abundant molecular and genetic tools of *Drosophila melanogaster* to investigate mechanisms of extinction following Pavlovian "fear" learning. We have established a robust assay of extinction (Fig. 4) and have tested a series of the classic learning mutants for effects on extinction. The surprising result from this analysis is that we observed a robust extinction learning in mutations that disrupt either

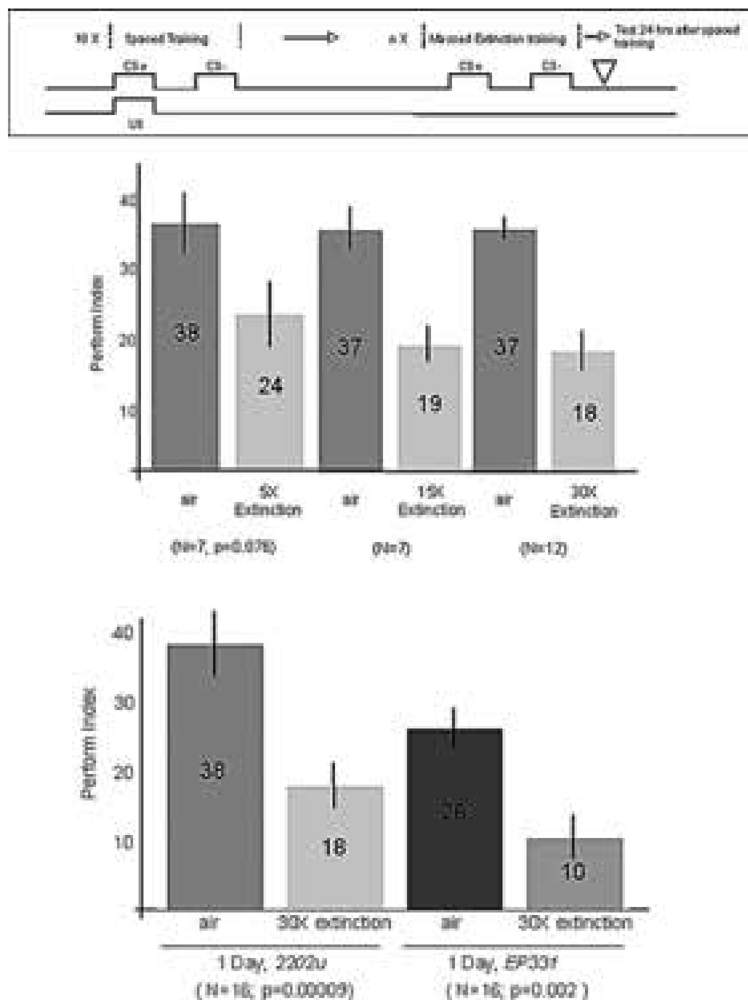

FIGURE 4 Extinction of "fear" conditioning in *Drosophila*. Extinction paradigm (*top panel*) involves three stages. First, animals are trained in a Pavlovian olfactory avoidance assay using ten sessions of spaced training. This leads to a robust and stable long-term "fear" memory. Second, 24 h after training, animals are subjected to varying numbers of extinction learning sessions that consist of repeated exposure to the odors in that absence of electric shock reinforcement. Finally, the animals are tested for avoidance of the conditioned odor. Using 5, 15, or 30 sessions of extinction, we observed a robust extinction of the fear avoidance (*middle panel*). Control groups receive exposure to air only. Our genetic investigations of extinction show that this form of learned safety is resistant to disruption of key signaling pathways with established roles in synaptic plasticity. These include cAMP signaling (not shown) as well as NMDA-R1 (*bottom panel*). NMDA-R1 mutant animals (EP331) display significantly reduced fear memory to begin with, but this residual memory can be extinguished robustly (compared with wild-type 2202U animals).

cAMP signaling or *N*-methyl-D-aspartate (NMDA) receptor function. We also observed extinction in mutations that disrupt either short-term or long-lived memory. In contrast, genetic disruption of the development of a single neural cell type appears to block extinction (Qin and Dubnau, in prep.). These results establish an entry point to dissect the neural circuits that serve fear conditioning versus extinction.

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STEM CELLS, SIGNAL TRANSDUCTION, AND BRAIN FUNCTION

G. Enikolopov M.-T. El-Daher J.-H. Park
J.M. Encinas N. Peunova
T. Michurina

The focus of our research is on stem cells in the adult organism and signaling molecules that control their self-renewal and fate. Our main focus is on stem cells of the adult nervous system. These cells retain the ability to produce new neurons throughout adult life, a function that may be important for learning and memory, mood regulation, and brain repair. We use animal models to study the fate of neural stem cells and to learn how different physiological stimuli—aging, disease, and therapies—affect stem cells of the adult brain. We also study stem cells of other tissues, with the goal of finding commonalities in the decisions that different types of stem cells make on whether to stay quiescent, produce progeny, or differentiate. We also study the signals that mediate interactions between stem cells and their microenvironment, with particular attention to the versatile signaling molecule nitric oxide (NO). Finally, we are trying to translate the knowledge that we generate with animal models to human studies.

DEFINED STAGES OF THE NEUROGENIC CASCADE IN THE ADULT HIPPOCAMPUS

New neurons are continuously generated in the hippocampus. They are born from neural stem cells, after proceeding through a cascade of events that include symmetric and asymmetric divisions, exit from the cell cycle, programmed elimination, and continuous changes of morphology; this cascade culminates with the young neuron establishing connections with other cells and becoming integrated into the preexisting neuronal circuitry. The generation of new neurons from stem cells is a highly dynamic process. It responds to a wide range of intrinsic and extrinsic factors that can enhance or suppress neurogenesis and may affect any step of the differentiation cascade. We aim to define each step of the cascade and determine the targets of important pro- or antineurogenic stimuli. Our main approach is to generate transgenic reporter animal lines, use them to dissect the neurogenesis cascade into distinct stages, and then identify the steps that are specifically affected by a particular stimulus. We have now combined a new approach for labeling dividing cells that greatly increases the resolution of the cell cycle analysis, lineage-tracing techniques, phenotyping progenitor cell populations, and computational modeling, to identify all of the major proliferative steps in the life cycle of stem and progenitor cells in the adult hippocampus. This allowed us to present a new scheme of the neuronal differentiation cascade in the hippocampus (Fig. 1); this scheme will now serve as a general platform upon which we and other researchers will be able to project the action of any agent that alters neurogenesis. We used this scheme to identify the targets of various antidepressant drugs and treatments, exercise, radiation, and chemotherapeutic agents and to address the mechanisms that control the quiescence, self-renewal, differentiation, and death of stem cells in the adult and aging brain.

DIVISION-COUPLED ASTROCYTIC DIFFERENTIATION OF NEURAL STEM CELLS: A NEW MODEL FOR ADULT HIPPOCAMPAL NEUROGENESIS

Production of new neurons in the hippocampus dramatically decreases with age, and this decline may underlie age-related cognitive impairment. The basic mechanisms of the age-related decline in hippocampal neurogenesis are not known. We used reporter transgenic lines, lineage tracing, pulse-chase labeling, and computational modeling to demonstrate that the main mechanism driving the age-related decrease in hippocampal neurogenesis is the disappearance of neural stem cells via their conversion into hippocampal astrocytes and that the disappearance of the stem cells is linked to their division (collaboration with Dr. Alex Koulakov at CSHL). In contrast to the conventional model of continuously self-renewing stem cells that undergo repeated cycles of division and quiescence, our results show that upon exiting their quiescent state, adult hippocampal stem cells rapidly undergo a series of asymmetric divisions to produce symmetrically dividing progeny, then exit the last cell cycle, convert into astrocytes, and leave the pool of stem cells; the gain in astrocytes is the result of the loss in stem cells. Thus, the decrease in the number of neural stem cells in the hippocampus is a division-coupled process and is a direct consequence of their functioning to produce new neurons. We now present a new scheme of the neurogenesis

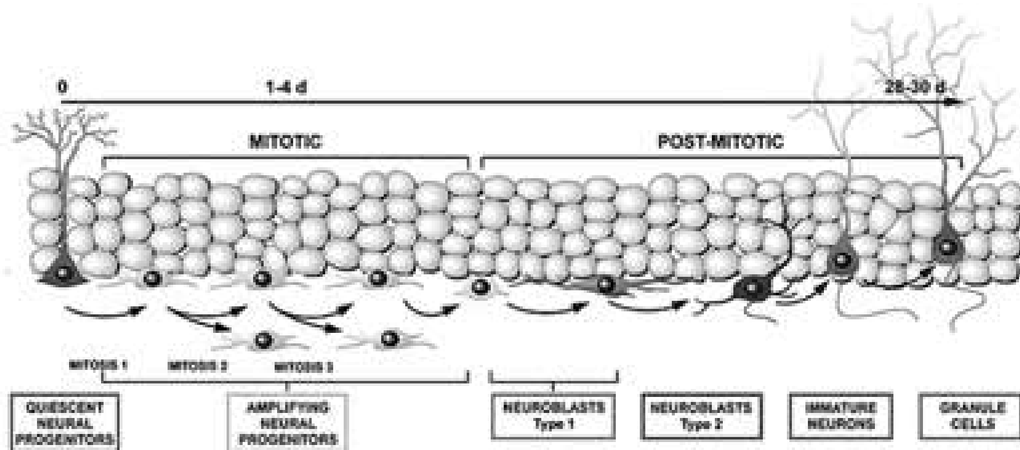

FIGURE 1 Neurogenic cascade in the dentate gyrus.

cascade in the adult hippocampus. We also propose a "disposable stem cell" model to explain the disappearance of hippocampal neural stem cells, the appearance of new astrocytes, the remodeling of the neurogenic niche, and the age-related decline in the production of new neurons.

NEUROGENIC TARGETS OF ANTIDEPRESSANT THERAPIES

Emerging evidence indicates a close link between the action of a diverse range of antidepressant therapies and augmented generation of new neurons in the adult hippocampus. Moreover, recent findings suggest that adult hippocampal neurogenesis not only accompanies but is required for the behavioral effects of antidepressants. We used the information on the stages of the neuronal differentiation cascade (above) to determine the cell populations targeted by various antidepressant therapies in the adult and developing brain. We aim to determine whether different types of treatments that regulate mood converge on the same step in the cascade or if each has their own target populations, to determine the changes in division/differentiation cascades induced by these treatments and to identify signaling molecules that translate the treatments into an increased number of new neurons. We have now determined the neurogenic targets of antidepressant drugs, electroconvulsive shock, deep brain stimulation, physical exercise, cosmic radiation, and aging. We are now using this information and the model of stem cell quiescence that we have developed (above) to identify signals that control the quiescence and self-renewal of stem cells, which direct the differentiation cascade in the adult brain and which can be used to repair the damaged and aging brain.

TRACKING NEUROGENESIS IN THE LIVE BRAIN

Changes in neurogenesis are associated with a host of conditions, including psychiatric and neurological disorders. A noninvasive method would be of key importance for harnessing the full potential of neural stem cells in health and disease; however, present imaging technologies cannot be applied for identification of neural stem cells in the live human brain. Recently, in a collaborative effort with scientists at Stony Brook University and Brookhaven National Laboratory, we developed a new approach, based on proton magnetic resonance spectroscopy (^{1}H-MRS), for detecting stem and progenitor cells in the live human and animal brain (Manganas et al., *Science 318:* 980 [2007]). Our findings open the possibility of investigating the role of stem cells and neurogenesis in a wide variety of human brain disorders and evaluating the efficiency of therapeutic interventions.

In an important further advance, our current collaboration with Dr. Helene Benveniste (Brookhaven National Laboratory) resulted in development of a new signal-processing protocol based on a commercial program developed specifically for the automatic quantitation of in vivo proton magnetic resonance (MR) spectra and is thus easier to reproduce. We have already used this approach to follow the changes in the live brain after electroconvulsive shock, combined with double S-phase analysis of cell division. These experiments confirmed our previous data, generated with a different signal-processing protocol, thus further validating the approach. They also pointed to additional markers of neurogenesis and helped to correlate the MRS signal with a specific division phase of the neurogenesis cascade. Importantly, these new results produced a robust experimental framework for detecting neurogenesis in the live brain. We will now

apply this approach as a powerful noninvasive method for studying the role of neurogenesis in the live animal and human brain.

STEM CELLS IN NONNEURAL TISSUES

Adult tissues undergo continuous cell turnover in response to stress, damage, or physiological demand. New differentiated cells are generated from dedicated or facultative stem cells or from self-renewing differentiated cells. Adult stem cells are often morphologically unspecialized, can survive for a long time or undergo long-term self-renewal, and are located in specialized niches that restrict their division and support their undifferentiated status. A number of approaches for identification and isolation of adult stem cells include immunophenotyping, using their label-retaining or drug-excluding properties, and selective marking. We generated a series of reporter transgenic mouse lines where stem and progenitor cells are marked by fluorescent proteins (including GFP, CFPnuc, H2B-GFP, DsRedTimer, and mCherry), driven by the regulatory elements of the nestin gene. Our original goal was to mark stem cells of the developing and adult nervous system, but we soon realized that the same reporter transgene marks stem and progenitor cells in a range of other organs and tissues, including nonneuronal tissues. The list now includes as dissimilar structures as brain and spinal cord, hair follicles, liver, pancreas, skeletal muscle, testis, ciliary margin of the eye, and anterior pituitary. This observation points to a close (and yet unexplained) relationship between expression of nestin and stem-like properties of cell populations in these tissues. It also provides a means of isolating adult stem and progenitor cells, tracing their lineage, and studying the mechanisms controlling their quiescence, division, self-renewal, and differentiation.

We recently used the nestin-expressing feature to identify and isolate stem cells of the adult anterior pituitary, a key regulator of the endocrine balance in the adult organism (collaboration with Dr. Geoffrey Rosenfeld, University of California, San Diego). We detected nestin-expressing cells in the perilumenal region of the mature anterior pituitary and, using genetic inducible fate mapping, demonstrated that they correspond to stem cells and serve to generate subsets of all six terminally differentiated endocrine cell types of the pituitary gland. Moreover, we found that similar stem-like undifferentiated cells are present in pituitary carcinomas and may represent cancer stem cells.

When following the lineage of adult stem cells of the anterior pituitary, we discovered that their role in the adult gland reflects a novel strategy for tissue maintenance, distinct from the strategies used by dedicated stem cells (such as hematopoietic stem cells in the bone marrow, stem cells in the bulge of the hair follicle, and neural stem cells in the neurogenic regions of the adult brain), by facultative stem cells (such as oval cells of the liver), or by dividing differentiated cells (such as hepatocytes and pancreatic β cells). In the pituitary, adult stem cells arise as a separate lineage, do not have a role in embryonic development, and contribute minimally to the pituitary of the newborn; however, they start functioning soon after birth, colonizing the organ that initially entirely consisted of differentiated cells derived from embryonic precursors and contributing prominently to the adult gland. As a result, adult anterior pituitary becomes a mosaic organ with two phenotypically similar subsets of endocrine cells that have different origins and different life histories. These parallel but distinct lineages of differentiated cells in the gland may help the maturing organism to adapt to changes in the metabolic regulatory landscape. We are now investigating whether the contribution of adult pituitary stem cells is modulated by strong physiological and pathological stimuli.

NO, STEM CELLS, AND DEVELOPMENT

Much of our interest is related to a versatile signaling molecule, nitric oxide (NO), a crucial regulator of vasodilation, immunity, and neurotransmission. We found that in several developmental and differentiation contexts, NO suppresses cell division, thus helping to control the balance between proliferation and differentiation. It thus arises as a regulator of stem cell activity in a range of tissues. For instance, it acts as a negative regulator of cell division in the developing and adult nervous system, such that by manipulating NO levels, we can change the number of neural stem and progenitor cells. Furthermore, we found that the neuronal NO synthase isoform (nNOS), usually associated with brain function, regulates hematopoiesis in vitro and in vivo. nNOS is expressed in adult bone marrow and fetal liver and is enriched in stromal cells, and there is a strong correlation between expression of nNOS in a panel of stromal cell lines and the ability of these cell lines to support hematopoietic stem cells; NO donors can further increase this ability. Moreover, hematopoietic progenitor cells of the bone marrow and spleen are regulated by nNOS, and in the nNOS knockout line that we generated, we found an increased number of these progenitors. These findings extend the role of nNOS beyond its action in the brain and suggest a model where nNOS, expressed in stromal cells, produces NO that acts

as a paracrine regulator of hematopoietic stem cells.

We continue to discover the diversity of the biological functions of NO. We are getting particularly interested in its role as a regulator of morphogenesis during early development. We found recently that during early development, NO coordinates two major morphogenetic processes: cell division and cell movement. Inhibition of NO synthase during early development of *Xenopus* increases proliferation in the neuroectoderm and inhibits convergent extension in the axial mesoderm and neuroectoderm, indicating that during development, NO suppresses division and facilitates movement of cells. We further found that NO controls these processes through two separate signaling pathways. Both of these pathways rely on RhoA-ROCK signaling but can be distinguished by the involvement of either guanylate cyclase or the planar cell polarity regulator Dishevelled. Concurrent control by NO helps to ensure that the crucial processes of cell proliferation and morphogenetic movements are coordinated during early development.

We have discovered yet another role of NO and nNOS during early development: their function as regulators of planar polarity of cilia. We found that in the *Xenopus* embryo, cilia gradually acquire a planar distribution and that suppression of XNOS1 (an ortholog of nNOS) activity destroys this planarity. We then determined the key partners of XNOS1 and found a new mode of establishing planar polarity by function-dependent stabilization of cilia growth. In addition to revealing a new role for NO, these findings highlight developing *Xenopus* as a model for the studies of mucosecretory epithelium, critical for human health and disease.

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MOLECULAR ANALYSIS OF NEURONAL RECEPTORS AND ION CHANNELS

H. Furukawa E. Karakas
N. Simorowski

The research in my group is aimed at understanding the molecular basis of cellular signal transduction pathways that are triggered and regulated by cell surface receptors, ion channels, transporters, and intramembrane enzymes. In particular, we elucidate the molecular mechanisms underlying neurotransmission and neurodegenerative diseases in the brain. Toward this end, we conduct structural and functional studies on neurotransmitter receptors, ion channels, transporters, and intramembrane enzymes, which mediate and regulate the strength of neurotransmission. To achieve our goals, we use X-ray crystallography to determine the three-dimensional structures of target proteins and test structure-based mechanistic hypotheses by site-directed mutagenesis in combination with biochemical and biophysical techniques including electrophysiology. Our group is currently focused on understanding the structure and function of two classes of calcium channels: the *N*-methyl-D-aspartate receptor (NMDAR) and the calcium homeostasis modulator (CALHM), dysfunctions of which are highly implicated in the pathogenesis of Alzheimer's disease.

STRUCTURE AND FUNCTION OF NMDARs

NMDARs are multimeric ligand-gated ion channels composed of NR1 and NR2 subunits that bind glycine and L-glutamate, respectively (Dingledine et al., *Pharmacol. Rev. 51:* 7 [1999]). The four distinct NR2 subunits, designated A through D, control ion channel properties, particularly the speed of deactivation kinetics, and show differential spatial and temporal expression patterns in specific neuronal circuits (Monyer et al., *Neuron 12:* 529 [1994]). The large extracellular region of NMDARs is composed of an amino-terminal domain (ATD) (Paoletti and Neytin, *Curr. Opin. Pharmacol. 7:* 39 [2007]) and a ligand-binding domain (S1S2) that bind allosteric modulator compounds and neurotransmitter ligands, respectively. The aim of our research is to determine the molecular structures of the extracellular region of NMDARs to understand how subtype specificity is defined through the architectures of S1S2 and how binding of modulators such as zinc to ATD can down-regulate ion channel function.

MECHANISM OF SPECIFICITY AND SLOW DEACTIVATION IN NR2D-CONTAINING NMDARs

We established an expression/purification protocol to obtain a sufficient amount of NR2D proteins in 2007. In 2008, we crystallized and solved the structures of NR2D S1S2 in complex with various ligands including L- and D-glutamate, aspartate, NMDA, and NHP5G. The structure revealed the molecular elements that define NMDA-subtype specificity and the pattern of protein conformational variations between the L-glutamate-bound form and the rest of the receptor-ligand binary complexes (Fig. 1). Using patch-clamp recording, we found that the effect of slow deactivation, which is a hallmark of NR2D function, is elicited by L-glutamate but not by other agonists. For that reason, we have hypothesized that the protein conformation seen in the structure of NR2D S1S2 in complex with L-glutamate represents the slow deactivation state of the receptors. We are currently testing this hypothesis by designing and conducting structure-based mutagenesis around the region of the conformational variability to further understand the molecular mechanism for slow deactivation in NR2D-containing NMDARs.

MECHANISM OF ZINC AND PHENYLETHANOLAMINE INHIBITION THROUGH THE AMINO-TERMINAL DOMAIN

In 2007, we initiated an extensive expression screening on ATDs of different subunits of the NMDARs and found that rat NR2B ATD can be expressed in insect cells in a sufficient amount for crystallographic studies. In 2008, we succeeded in optimizing an expression and purification protocol for NR2B ATD, crystallizing the proteins and solving the crystal structures in apo and zinc-bound states. The current crystallographic study revealed the overall architecture of the ATD for the first time and pinpointed the zinc-binding sites (Fig. 2). The structure has also shown a plausible mechanism by which phenylethanolamine compounds such as ifenprodil may specifically bind to NR2B ATD.

One interesting observation is the presence of a subunit association with a potential physiological implica-

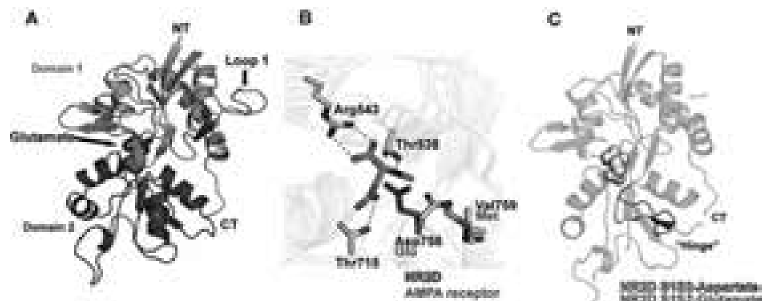

FIGURE 1 Crystal structure of NR2D S1S2. (*A*) Crystal structure of NR2D S1S2 in complex with L-glutamate. (*B*) Ligand-binding site in complex with NMDA. The NMDA molecule binds to NR2D through electrostatic interaction and hydrogen bonds (dots). (*C*) The two distinct conformations of "hinge" for aspartate-bound and glutamate-bound forms. The conformations of NMDA-bound and pNHP5G-bound forms are identical to the L-aspartate-bound form.

tion. We are currently designing functional experiments to validate the physiological relevance of the NR2B ATD dimer in the context of full-length NMDARs. Another interesting observation is that there are multiple zinc-binding sites within the NR2B ATD structure. We are conducting site-directed mutagenesis of the zinc-binding sites in combination with electrophysiology to determine which zinc sites mediate allosteric inhibition of ion channel activity. We will continue with our effort to understand the mechanism by which bindings of zinc, phenylethanolamines, and protons to NMDARs inhibits ion channel activities by using a combination of X-ray crystallography, biochemistry, and electrophysiology.

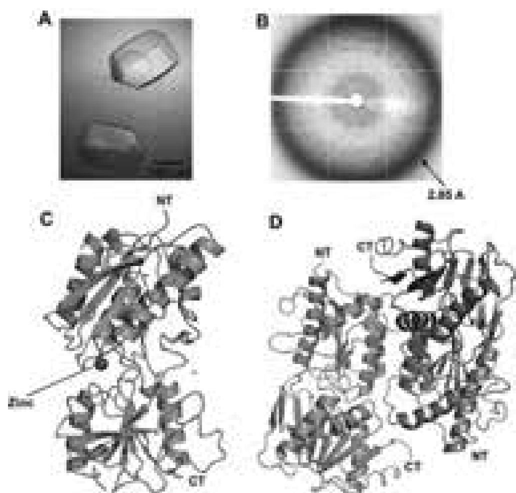

FIGURE 2 Crystal structure of NR2B ATD at low pH. Hexagonal crystals of NR2B ATD (*A*). The crystals diffracted the X-ray to ~2.85 Å (*B*). Preliminary crystal structure solved by the single anomalous diffraction method. The NR2B ATD has a bilobed structure with amino and carboxyl termini (NT and CT) located at the opposite ends (*C*). The NR2B ATD protomers form a dimer in the crystal (*D*).

STRUCTURE AND FUNCTION OF THE CALCIUM HOMEOSTASIS MODULATOR

Calcium homeostasis in neuronal synapses is maintained by cell surface and endoplasmic reticulum (ER) membrane proteins including NMDARs, voltage-gated calcium channels, IP3 receptors, and the recently identified *cal*cium *h*omeostasis *m*odulator proteins (CALHM) (Dreses-Werringloer et al., *Cell 133:* 1149 [2008]). Spatiotemporal regulation of Ca^{2+} concentration is pivotal for brain physiology including learning and memory formation. Disruption of calcium homeostasis has long been suggested to cause neurodegenerative diseases including Alzheimer's disease (AD). CALHM, identified in 2008, is the first Ca^{2+}-regulating protein whose allelic polymorphism is directly linked to late-onset AD. Currently, there is little or no knowledge on the functional properties of CALHM. For example, there is no functional evidence showing what triggers the opening of CALHM. Thus, by using biochemical, electrophysiological, and structural biological approaches, we are dissecting the function of CALHM at a molecular level.

PRECRYSTALLIZATION SCREENING AND CHARACTERIZATION OF CALHM

Structural study of eukaryotic integral membranes remains a field with limited success due to difficulties in acquiring a sufficient amount of functional protein samples suitable for crystallographic studies. To increase the

likelihood of obtaining crystals for X-ray crystallographic study, it is critical to examine the size homogeneity of protein samples before crystallization. The expression profile and size homogeneity of the proteins depend on the nature of amino acid sequences. For that reason, it is advantageous to assess the properties of protein constructs derived from various species with sequence variability. My laboratory uses a green fluorescence protein (GFP)-based precrystallization screening method called fluorescence-coupled size-exclusion chromatography (FSEC) to quickly analyze cDNAs from various species for protein expression and size homogeneity. In this method, cDNAs encoding proteins of interest are fused to GFP and expressed in mammalian, insect, and yeast cells. The GFP fusion proteins, solubilized in a detergent such as dodecyl maltoside, are subjected to a size-exclusion chromatography and detected by GFP fluorescence, instead of conventional ultraviolet (UV) absorbance. This allows the candidate proteins to be assayed for size homogeneity without a need for large-scale expression and purification.

We are currently subjecting various subtypes of CALHM cDNAs from *Caenorhabditis elegans*, mouse, rat, zebrafish, chicken, and humans to FSEC precrystallization screening to find a CALHM protein amenable to a crystallographic study.

CONSTRUCTION AND FUNCTION OF THE GABA INHIBITORY CIRCUITS

Z.J. Huang Y. Fu, M. He, D.K. Krishnan, S. Kuhlman, D. Kvitsiani, M. Lazarus, J. Lu, A. Paul, R. Pike, H. Taniguchi, P. Wu, X. Wu

In many areas of the mammalian brain, GABAergic interneurons are crucial in establishing the functional balance, complexity, and computational architecture of neural circuits. GABA-mediated inhibition regulates synaptic integration, probability, and timing of action potential generation. Furthermore, interneurons generate and maintain network oscillations that provide temporal structures for orchestrating activities of neuronal populations. The rich variety and fine details of inhibition are achieved by diverse interneuron cell types that display distinct morphology, physiological properties, connectivity patterns, and gene expression profiles. The biophysical properties of different cell types are optimized for generating a rich array of firing patterns and synaptic dynamics to precisely control electrical signaling in neural networks. Furthermore, interneurons display class-specific axon arbors and innervation patterns that distribute their outputs to discrete spatial locations, cell types, and subcellular compartments in the network. Like glutamatergic connections, GABAergic synapses are also modified by usage, leading to reconfiguration of the inhibitory circuitry by experience. Understanding the organization, function, and plasticity of GABAergic interneurons is key to discovering the general principles that govern how information is processed by neural circuits.

TOWARD A GENETIC DISSECTION OF THE GABAERGIC SYSTEM

For decades, the heterogeneity of cell types have been a major challenge to study the GABAergic system using conventional anatomical and physiological techniques. The genetic approach is ideal to dissect the complexity of the GABA system by tapping into the intrinsic gene regulatory mechanisms that generate and maintain the diverse cell types. Using mouse genetic engineering (e.g., gene targeting, bacterial artificial chromosome [BAC] transgenics, and Cre/loxP recombination-regulated gene expression), we are systematically building genetic tools for studying GABAergic neurons. We are generating ~20 knockin “driver lines” expressing Cre or inducible CreER to establish “genetic access” to all major classes of GABAergic neurons. These driver lines will allow us to (1) visualize the morphology and connectivity of GABA neurons with synapse resolution, (2) monitor their activity and plasticity, and (3) manipulate their function. Our initial characterization of several lines showed that they gave expected recombination patterns that recapitulated the endogenous cell-type-specific markers. In collaboration of Dr. Sacha Nelson at Brandeis University, we are characterizing these driver lines and establishing a web-based platform for disseminating mice, reagents, and expression data (UO1-MH078844-01; NIH Neuroscience Blueprint). These driver mice will significantly accelerate progress in studying all aspects of the GABAergic system.

We recently developed a general and powerful method that combines Cre-driver mice and virus-mediated gene expression to label and manipulate specific neuron types in mice (Kuhlman and Huang 2008). We engineered adeno-associated viruses (AAVs) that express green fluorescent protein (GFP), dsRedExpress, or channelrhodopsin (ChR2) upon Cre/loxP recombination-mediated removal of a transcription–translation STOP cassette. When applied to the Pv-Cre mice, this method allowed two-photon imaging of cortical basket cells and perisomatic synapses in live mice. Genetic access to specific cell types through Cre driver mice combined with efficient delivery of conditional viral vectors to visualize and manipulate these cell types will herald a new era for studying the organization and function of mammalian neural circuits.

MOLECULAR MECHANISMS UNDERLYING SUBCELLULAR ORGANIZATION OF GABAERGIC SYNAPSES

Large principal neurons, such as cortical pyramidal neurons and cerebellar Purkinje neurons, consist of distinct subcellular domains (spines, dendrites, soma, axon initial segments, etc) that allow distributed and compartmentalized signal processing and increase the computational power of single neurons. Superimposed

upon these anatomical and physiological compartments is the subcellular organization of synaptic connectivity. In particular, subcellular organization of inhibitory synapses is essential for regulating synaptic integration, spike timing, and back propagation in principal neurons. Until recently, the mechanisms underlying subcellular synapse organization were almost entirely unknown. We have demonstrated that subcellular targeting of GABAergic synapses is guided by genetically determined mechanisms and does not involve experience-dependent neural activity (Di Cristo et al., *Nature Neurosci. 10:* 1569 [2007]). Furthermore, we discovered that the subcellular distribution of an L1 family immunoglobulin protein (neurofascin186), recruited by an ankyrin family membrane skeleton (ankG480), is a key mechanism that directs basket interneurons to innervate the axon initial segment of Purkinje neurons (Ango et al., 2008). Importantly, the L1CAM and ankyrin families consist of different members that localize to distinct subcellular compartments. We recently discovered that another member of L1CAMs, CHL1 (close homolog of L1), contributes to the innervation of Purkinje cell dendrites by stellate interneurons (Ango et al. 2008). Our findings support the general hypothesis that members of L1CAMs recruited to subcellular domains by different ankyrins contribute to subcellular organization of GABAergic synapses.

ACTIVITY-DEPENDENT MATURATION AND PLASTICITY OF INHIBITORY SYNAPSES AND CIRCUITS

The construction of neural circuits requires activity-dependent regulation of neurite growth and synapse formation. As key mediators of neural activity, neurotransmitters are particularly well suited to sculpt connections by coupling synaptic transmission with synaptic growth and refinement. Indeed, glutamate signaling regulates nearly all aspects of excitatory synapse development and plasticity. The mechanisms underlying activity-dependent development of inhibitory synapses and circuits are poorly understood. We have shown that the maturation of inhibitory synapses targeted to the soma and proximal dendrites of pyramidal neurons in visual cortex is regulated by neural activity and visual experience (Chattopadhyaya et al., *J. Neurosci. 24:* 9598 [2004]). We further discovered that GABA and its rate-limiting synthetic enzyme GAD67 act beyond their classic roles in inhibitory transmission—they also mediate activity-dependent maturation and plasticity of inhibitory synapses and innervation patterns (Chattopadhyaya et al., *Neuron 54:* 889 [2007]). We hypothesize that, similar to the role of glutamate in the development of excitatory synapses, GABA signaling is an activity-dependent mechanism that promotes and coordinates pre- and postsynaptic maturation during the development of inhibitory synapses. Because GAD67 level is coupled to neuronal inputs, activity-dependent GABA synthesis and signaling may provide a cell-wide as well as synaptic mechanism to sculpt GABAergic connectivity patterns. Our discovery thus provides major insights into the activity-dependent maturation and plasticity of inhibitory synapses. We are currently studying the underlying cellular and molecular mechanisms.

MATURATION OF INHIBITORY CIRCUITRY AND CRITICAL PERIOD PLASTICITY

Maturation of GABA inhibitory circuitry in primary visual cortex regulates the critical period of ocular dominance (OD) plasticity. We discovered a role for polysialic acid (PSA), presented by the neural-cell-adhesion molecule (NCAM), in the maturation of GABAergic synapses, inhibitory transmission, and OD plasticity. Our results suggest that developmental and activity-dependent removal of PSA is a permissive mechanism that regulates the timing of the maturation of GABA inhibition and onset of OD plasticity (Di Cristo et al., *Nature Neurosci. 10:* 1569 [2007]).

The cellular mechanisms by which different classes of inhibitory interneurons regulate OD plasticity are unknown. The cell-type-specific Cre drivers and Cre-regulated viral vectors that we have developed provide an unprecedented opportunity to address this important question. By targeted multiple patch-clamp recording of genetically labeled interneurons in visual cortical slices, we are characterizing the functional maturation of specific classes of interneurons during the critical period. By manipulating inhibitory transmission in defined classes of interneurons in vivo, we will be able to unravel their specific contribution to OD plasticity and the critical period.

GENE EXPRESSION PROFILES IN GABAERGIC CELL TYPES

We have initiated an effort to characterize gene expression profiles in several classes of GABAergic interneurons using a procedure to manually purify GFP-labeled interneuorns from our BAC transgenic reporter mice (Sugino et al., *Nature Neurosci. 9:* 99 [2006]). We are further developing efficient methods to achieve cell-

type-based gene expression profiling in the brain. A systematic understanding of gene expression profiles among different classes of interneurons during development and in response to stimulus will yield fundamental insight into the genetic logic underlying the construction and function of the GABAergic system.

GABAergic Interneurons and Neurodevelopmental Disorders

Genetic and epigenetic perturbations of the GABAergic system results in altered development, function, and maladaptive plasticity of neural circuits, aberrant information processing, and cognitive deficits in a variety of psychiatric disorders such as schizophrenia (SZ) and autism. We have received major funding from the Simons Autism Research Initiative to study Rett Syndrome (RTT), the best-characterized form of the autism spectrum disorders, caused by mutations in the X-linked gene *MeCP2*. Our general hypothesis is that *MeCP2* mutations perturb the postnatal maturation of the connectivity, function, and plasticity in subsets of inhibitory interneurons in distributed brain areas, leading to altered development, maladaptive plasticity of neural circuits, and characteristic behavioral deficits. In collaboration of Dr. Sacha Nelson at Brandeis University, we combine genetic, genomic, imaging, and physiological approaches to (1) identify the subset of GABAergic neurons in specific brain regions that are influenced by *MeCP2* mutations at defined developmental stages, and during experience-induced plasticity; (2) characterize the impact of mutations on gene expression profiles in specific classes of neocortical interneuron; and (3) determine the impact of mutations on the physiological properties and synaptic innervation pattern of specific classes of neocortical interneurons.

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Priscilla Wu

NEURAL CIRCUIT DYNAMICS UNDERLYING DECISION MAKING

A. Kepecs G. Costa S. Ranade
D. Kvitsiani J. Sanders

Research in our laboratory focuses on the neurobiological mechanisms and computational principles of decision making. We view complex behaviors as sequences of elementary decisions that can be conveniently studied in isolation to expose their underlying neural processes. Yet even simple decisions involve the integration of sensory and memory information with emotional and motivational attributes requiring the concerted action of millions of neurons across brain regions. Therefore, we take an integrative approach, combining tightly controlled rodent behavior with electrophysiological recordings to monitor neural activity, molecular tools to perturb genetically identified neural circuit elements, and quantitative analysis to guide and sharpen the neurobiological questions.

At present, we are pursuing two broad directions. First, we seek to understand the principles of decision making and, in particular, how confidence estimates are computed and used by the brain. Recently, we discovered neural correlates of decision confidence in the orbitofrontal cortex of rats, and we are currently investigating both the neural mechanisms and the algorithms by which this signal supports adaptive behavior. Second, we want to understand how specific cell types participate in the neural circuit dynamics of local processing and how different brain regions with specialized functions coordinate their activity. Toward these goals, we are adapting molecular and optical tools for use in behaving mice to identify and manipulate defined neuronal subtypes and pathways.

Ultimately, we would like to gain mechanistic insights into decision processes in the hope that these will be of utility not only for a basic scientific understanding, but also for better treatments of brain dysfunctions of decision making, such as pathological gambling, drug abuse, and anxiety disorders.

Behavioral Tasks to Study Confidence Judgments

G. Costa, A. Lak [in collaboration with Z.F. Mainen, Instituto Gulbenkian de Ciêcia (IGC) and Champalimaud Foundation]

If you are asked to evaluate your confidence in something you know—how sure are you that it is true—you can readily answer. What is the neural basis for such judgments? Is knowledge about beliefs an example of the human brain's capacity for self-awareness? Or is there a simpler explanation that might suggest a more basic yet fundamental role for uncertainty in neural computation? To study these questions, we are developing new behavioral tasks.

Our goal was to establish variants of our well-studied olfactory categorization task, in which an estimate of decision uncertainty or confidence about the original decision is turned into a behavioral action. We first sought to develop a task for measuring confidence behaviorally on a trial-by-trial basis in order to examine the neural mechanisms of confidence judgments. To manipulate confidence, we used an olfactory mixture categorization task to systematically vary decision uncertainty by changing the ratio of the two components in the odor mixture. To measure confidence, we delayed reward delivery and then measured the time an animal was willing to wait at the reward ports. In incorrect trials (which were only signaled by the lack of reward delivery), we noticed that rats stayed longer following difficult (uncertain) decisions than after easier (certain) decisions. To extend these observations to correct choices, we introduced a small fraction of "catch" trials for which reward was omitted. In these omitted reward trials, rats waited longer on average than in incorrect trials. To make these waiting time estimates robust, we optimized the parameters of this task to maximize the differences in waiting time for different levels of uncertainty. In this new task, the duration the rats are willing to wait at the reward port reflects decision uncertainty. Therefore, this task will allow us to examine how the timing of the decision to leave the reward port ("leaving decision") depends on the uncertainty about the original decision. This will allow us to study how access to an internal measure of decision confidence is used in an adaptive behavior and to examine the nature of the neural processes underlying decision confidence.

Causal Role of Orbitofrontal Cortex in Confidence-guided Decisions

A. Lak, G. Costa [in collaboration with Z.F. Mainen, IGC]

We previously discovered neurons in the orbitofrontal cortex (OFC) that signal decision confidence. We are

pursuing these initial observations by trying to establish that confidence-related neural activity in the OFC is *causally related* to confidence judgments.

We hypothesized that shutting off the OFC leaves the odor-guided decision intact while impairing the use of confidence-guided leaving decisions. Therefore, we bilaterally implanted double cannulae to temporary block neural activity in the lateral and ventrolateral portions of the OFC. We measured rats' confidence using the waiting time version of our olfactory categorization task discussed above. On testing days, rats received an intra-OFC infusion of muscimol (a GABAergic agonist that silences neural activity) or a saline control solution. Our preliminary results show that the waiting time patterns of the OFC-inactivated animals are disrupted without affecting olfactory discrimination performance. We are currently optimizing the inactivation methods and cannula positions to confirm these results.

These preliminary results are consistent with studies of human patients with orbitofrontal lesions who are unable to judge their degree of uncertainty, indicating that the functions of the OFC that we are investigating in rats are conserved across species. By establishing that the OFC is a causal contributor to a confidence-guided decision, we hope to lay a strong foundation for a broader examination of the neural mechanisms underlying confidence estimation.

Active Learning in Rats Uses Uncertainty Estimates

A. Kepecs [in collaboration with N. Uchida, Harvard University and Z.F. Mainen, IGC]

Statistical learning theory proposes that "active learners" use their uncertainty estimates to optimally set their learning rate so as to learn more when uncertain and less when certain. In the machine-learning literature, it is well established that active learning based on informative datapoints speeds up learning. However, little is known about whether these principles apply to animal learning. We tested the hypothesis that rats are active learners and use uncertainty estimates to learn optimally.

To study this issue, we used an odor-mixture categorization task performed by rats and examined the trial-by-trial updating of behavioral strategy. As animals learn to perform a categorization task, they use reinforcement feedback to establish the decision boundary, yet this boundary may be continually updated during ongoing performance after overt learning asymptotes.

Indeed, we found that animals dynamically adjust their decision strategy even after extensive training. For difficult decisions (those near the category boundary), the outcome is very informative about location of the decision boundary, whereas the outcome of pure odor trials (far from the decision boundary) reveals little about the boundary. Therefore, statistical principles suggest that the decision boundary should be adjusted more following difficult trials with high uncertainty than for trials with no uncertainty. Consistent with this, we found that rats biased their decisions toward the most recently rewarded direction, as if their decision boundary was shifted. Moreover, the magnitude of this bias was proportional to the uncertainty of the previous decision, as predicted. This bias, however, was only observed for difficult decisions, suggesting that the category boundary and not the side-bias, was being updated. Therefore, our results show that rats are "active learners," combining reward feedback and decision uncertainty estimates to update their decision strategy.

These findings establish a new learning phenomenon, in which animals appear to use their uncertainty to adjust their learning. Future studies will examine the neural basis of this active learning process.

Bayesian Classifier Models for Active Learning

A. Kepecs [in collaboration with P. Shenoy, E. Chastain, and R.P.N. Rao, University of Washington]

In the machine-learning literature, active learning is a well-established mechanism used to increase the rate of learning. Active learning mechanisms are computationally advantageous because they lead to the so-called "less is more" effect in which fewer samples lead to better classifiers. Our recent observations show for the first time that animals can be active learners concordant with statistical learning theory. Therefore, we sought to model the rats' behavior using machine-learning methods.

We applied a recent probabilistic interpretation of support vector machine (SVM) classifiers to explain the trial-by-trial updating of the decision boundary in a normative fashion. In Bayesian SVMs, the size of the margin for a sample provides the posterior likelihood that the decision was correct, i.e., a measure for the classifiability of the sample given the current model. The inverse of this is a measure of uncertainty. First, we noticed that this uncertainty measure shows the same characteristic patterns as the firing rates of OFC neurons. Second, our model reproduced the distinct behav-

iorally observed choice bias patterns shown by rats following correct and incorrect trials. The results provide a new interpretation of active learning in categorization tasks in terms of margins in Bayesian classifiers.

Sequence Processing in the Mouse Hippocampus

J. Sanders

We are able to recall lists of items and episodes of our lives, with the order of events appropriately preserved. When experiencing a known pattern of events, we can effortlessly sense when things are out of order. Our research aims to establish how the brain represents the order of sensory features at >1-s-long timescales. We focus on the CA1 region of the hippocampus as a candidate area for both real-time sequence prediction and order-violation detection.

Toward this end, we are developing auditory sequence tasks that a mouse can perform while head-fixed. In brief, a head-fixed mouse listens to a repeating pattern of unique 2-s-long sounds, for which only the final sound in the pattern is rewarded by water delivery. We find that during a week's training, the mouse licks faster in anticipation of the reward sound. To gain behavioral evidence that mice know the sequence, we used a task version in which a mouse is not allowed to lick for water except during the final sound in the pattern. In this case, we found a reaction time deficit for rare catch trials in which the reward sound alone is presented early in the sequence, compared with rare trials for which the final two sounds are presented early. At present, we are training mice in different sequence tasks that will provide better behavioral evidence for sequence prediction.

Our plan is to record neural activity in the hippocampal CA1 region while mice perform sequence tasks. We hypothesize that hippocampal neurons will represent abstract auditory sequences, generate sequence element predictions, and detect sequence violations. Why would that be interesting? Currently, it is believed that the human hippocampus is responsible for integrating multimodal information into spatiotemporally delimited episodic representations that underlie episodic memory. On the other hand, recordings from rodent hippocampus show "place cells" that represent unique positions in space. By showing that such "place cells" can signal abstract sequence information as well, we hope to link these two disparate sets of observations and open a new avenue for understanding hippocampal processing.

Interarea Communication Protocols in the Rodent Brain during Foraging

S. Ranade

The complex architecture of the mammalian brain can be compared to the internet with its multitude of nodes interconnected through an intricate network of wires. To effectively communicate and route information to the appropriate nodes, the internet uses common network protocols. Similarly, diverse brain areas must also coordinate their activity in a temporally precise manner to process diverse sources of sensory and memory signals in a coherent way for executing proper actions. We are exploring the hypothesis that oscillatory activity in the θ frequency range (4–12 Hz) can serve as the basis for a interbrain areal communication protocol.

Rats extensively use their sense of smell and touch (whiskers) as they navigate and explore their surroundings. During bouts of exploration, both sniffing and whisking occur rhythmically at characteristic frequencies within the θ range (4–12 Hz). Similarly, hippocampal networks oscillate in the same frequency range. Therefore, we asked whether these rhythms in peripheral sensory modalities, i.e., sniffing and whisking, are coordinated or independent and, if so, whether they are also coordinated with the hippocampal θ rhythm.

To address these questions, we simultaneously recorded sniffing (with a thermocouple), whisking (with mystachial EMG wires), and hippocampal θ rhythm (with microwires) in freely moving rats that were engaged in cued foraging. A sound cue signaled the availability of a food reward at random places in the arena. Rats engaged in bouts of intense exploratory activity involving sniffing and whisking as they navigated through the arena. We initially found that on a longer timescale, sniffing and whisking frequencies were not always correlated. However, we noticed that right around the presentation of the auditory cue, there was transient coherence between the two rhythms, and on some occasions, these sensory rhythms were also correlated with the hippocampal θ rhythm. Interestingly, the locking between sniffing and whisking was not always 1:1; rather, we observed multimode locking at 2:1, 3:1, or 1:2, such that the protraction phase of whisking was always coincident with the inhalation phase of sniffing.

Our initial findings thus suggest that although these distributed sensorimotor and subcortical rhythms are not always correlated, they can be transiently synchronized following a salient behavioral event. Therefore, θ rhythms may be used in interareal communication to establish a common carrier frequency. We are pursuing this question further by refining the foraging task to bet-

ter control the animal's behavioral state. We would also like to directly test whether synchronization of various rhythms at θ frequency results in better information transfer in terms of spiking activity in the hippocampus and primary sensory areas in the brain.

Developing New Spectral Variants of Channelrhodopsin-2

J. Sanders

Channelrhodopsin2 (ChR2), a light-gated cation channel from algae, is revolutionizing the way that we study neural circuits. A neuron expressing ChR2 will fire precisely timed action potentials in response to pulses of light, restricting stimulation to genetically defined cell types. Whereas multicolored variants of green fluorescent protein (GFP) have clear utility in studying gene expression patterns, variants of ChR2 that respond to different wavelengths of light would allow direct control and comparison of two distinct population of neurons or neural pathways.

We first BLAST-searched ChR2 against the J. Craig Venter Institute's Global Ocean Sampling Expedition database and found several full-length sequence fragments that have high homology with ChR2. From the most similar sequences in the database, we used homology with bacteriorhodopsin to eliminate sequences lacking the key residue for covalent bonding to retinal and other distinguishing features common to nonphotoreactive "opsin-like" protein families. Our remaining hits did not match known sequences, but they were most similar to opsin proteins in algae and dinoflagellates. We had full-length DNA fragments of these top hits synthesized and successfully cloned them into mammalian expression vectors. We also used structural homology with bacteriorhodopsin and literature on bacteriorhodopsin's known single-nucleotide "spectral switch" to synthesize a series of point mutants of ChR2, which we believe may have altered spectral selectivity. We created gene-gun bullets for two of ChR2's oceanic homologs and eight point-mutants. All of these constructs were successfully expressed cultured hippocampal slices and appeared to be inserted into cell membranes.

We plan to test these constructs by recording from *Xenopus* oocytes and hippocampal cells while scanning through different wavelengths of light. Any large shift in spectral selectivity or other desirable change in channel kinetics will warrant further characterization of the ChR2 variant in order to determine its usefulness as an optogenetic tool.

Neural Circuit Dynamics of Genetically Identified Neurons in Behaving Mice

D. Kvitsiani, S. Ranade [in collaboration with Z.J. Huang, Cold Spring Harbor Laboratory]

In vitro studies of neural circuits provide a picture of the brain with exquisite precision in neural connections between specific neural cell types. In contrast, successes in systems neuroscience have come from relating single-neuron activity with perception and behavior, without knowing the identity of the recorded neurons. Our goal is to bridge this gap by developing a method to determine the molecular identity of the neurons recorded during behavior in order to examine the neural circuit dynamics.

The availability of genetically targetable fluorescent markers and activity reporters has revolutionized cellular neuroscience, and similarly, the recently characterized ChR2, a light-sensitive cation channel, promises to do the same for systems neuroscience. Using ChR2, genetically defined neuron classes can be made light activatable so that one can optically control the generation of spikes with millisecond precision. To take advantage of these molecular tools, we are developing methods to simultaneously optically activate and electrophysiologically record genetically defined neuron types in behaving mice.

We managed to obtain a genetic system to label specific classes of interneurons with high efficiency and specificity. We used the mouse knockin line expressing Cre protein under the endogenous parvalbumin (PV) promoter in combination with adeno-associated virus (AAV) injections. We made AAVs that carry the ChR2-coding sequence under a strong ubiquitous promoter interspersed by a floxed transcriptional stop cassette (loxp-STOP-loxp). This way, we have managed to have a high and very specific expression of the ChR2 protein (>90% specificity) in hippocampal PV interneurons. We also made an additional mouse line that expresses Cre in calretinin-positive interneurons. In parallel, we also generated a reporter mouse line to express the ChR2 protein in a Cre-dependent manner. We inserted the ChR2-coding sequence preceded by a CAGGS promoter and the loxP-STOP-loxP sequence into the *Rosa26* locus. Therefore, in combination with any cell-type-specific Cre driver, we should be able to target ChR2 proteins in those specific neurons. These mouse lines are currently being characterized. This work was done in Josh Huang's lab.

We next performed pilot experiments in anesthetized animals to verify that we can optically stimulate and electrically record PV interneurons at the same time. We saw light-evoked responses in some neurons and local

field potential deflections that confirm the validity of our approach. This encouraged us to quickly move to freely behaving animals.

We designed a new “drive” that can house up to ten independently movable electrodes and/or fiberoptics. This new drive is lightweight (~4 g) and thus can be chronically implanted on the head of a mouse. We also designed custom-built fiber-optic connectors that can be mounted on the drive and pass light from external sources (LED or laser). Currently, these tools enable us to optically stimulate neurons and electrophysiologically record them at the same time in freely moving mice.

We aim to use these tools to understand how the diversity of hippocampal inhibitory neuron types underlies distinct neural circuit dynamics. Specifically, it is known that the hippocampus exhibits different states of activity organized into oscillations spanning multiple timescales. These neural population oscillations are known to change depending on a behavioral state of an animal—from active exploration to different sleep states. We hypothesize that PV interneurons regulate the θ rhythm (because they can directly inhibit excitatory principal cells), whereas CR interneurons regulate the γ rhythm (because they tend to inhibit other interneurons). Therefore, CR and PV interneurons might have complementary roles in controlling neural population rhythms. We plan to test the functional roles of these interneuron classes by characterizing their activity using optical tagging and stimulation to observe their impact on global neural activity and behavior.

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Adam Kepecs (left) and Duda Kvitsiani

THEORETICAL AND COMPUTATIONAL NEUROSCIENCE

A. Koulakov D. Tsigankov

Our laboratory develops theoretical models for various processes occuring in the brain. We work in parallel on three important topics. First, we have proposed a model for combining genetic information and experience (nature and nurture) during neural development. This model has been tested in simple circuits forming in the brain that can be modified genetically, surgically, and pharmacologically. Second, we have applied dimension-reduction techniques to the database of odor character profiles that contains vast amounts of information about human olfactory perception. The goal of this research is to find the organizing principle that can explain human olfaction. Third, we have been working on the mathematical description of adult neural stem cell differentiation and proliferation in hippocampus. These models can describe the evolution in time of various markers that experimental researchers use to study the division/differentiation cascade. By comparing the computational/theoretical models to experimental data, one can understand changes occurring in neurogenesis due to aging and antidepressant therapies.

Optimal Axonal and Dendritic Branching Strategies during Development of Neural Circuitry

D. Tsigankov, A. Koulakov

In developing brain, axons and dendrites are capable of connecting to each other with high precision. Recent advances in imaging allowed monitoring of the axon, dendrite, and synapse dynamics in vivo. It was observed that the majority of axon and dendrite branches formed are retracted later during the development. In this study, we computationally analyze different axonal and dendritic branching strategies that minimize the number of transient branches required to establish the connectivity with particular precision. We apply these branching rules to the development of retinotectal topographic connectivity and find that axons and dendrites have different optimal branching strategies. The axonal optimal strategy is to form new branches in the vicinity of existing synapses, whereas the optimal rule for dendrites is to form new branches preferentially in the vicinity of synapses with correlated presynaptic and postsynaptic electric activity. We show that an experimentally observed different reaction to the *N*-methyl-D-aspartate (NMDA) receptor block in the dynamics of axonal and dendritic branching implies that these two branching strategies are implemented in the developing brain. We suggest that the difference in branching strategies of axons and dendrites could be detected by measuring the spatial correlations between synapses and branch points on the developing arbors. We thus predict that these correlations should be reduced for dendrites but not for axons under the conditions of the NMDA receptor block.

The Structure of Human Olfactory Space

A. Koulakov [in collaboration with A. Enikolopov, Cold Spring Harbor Laboratory, and D. Rinberg, HHMI Janelia Farm]

We analyze the psychophysical responses of human observers to an ensemble of monomolecular odorants. Each odorant is characterized by a set of 146 perceptual descriptors obtained from a database of odor character profiles. Each odorant is therefore represented by a point in highly multidimensional sensory space. In this work, we study the arrangement of the odorants in this perceptual space. We argue that the odorants densely sample a two-dimensional curved surface embedded in the multidimensional sensory space. This surface can be approximated by the exterior of a spherical wedge (Fig. 1). We suggest that this two-dimensional surface represents the relevant manifold sampled by the human olfactory system, thereby providing a definition of olfactory sensory space. We also developed a computational method for relating the position of a particular odorant on the two-dimensional surface to its physicochemical descriptors. On the basis of this analysis, we conclude that one of the parameters describing the two-dimensional surface is correlated with the count of carbon atoms in the odorant molecule, whereas the other parameter is related to the molecule's polarity or hydrophobicity.

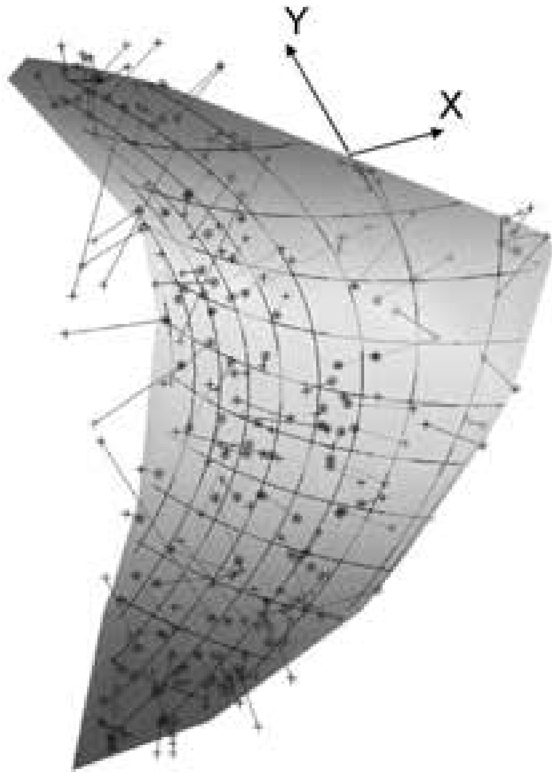

FIGURE 1 Structure of human olfactory space. The odors (crosses) cluster around a two-dimensional curved surface in the perceptual space. The two-dimensional surface exists in a 146 dimensional space of various perceptual descriptors. The parameters defining the positions on the surface (*X* and *Y*) are related to the molecule's chemical composition and hydrophobicity.

Disposable Tissue Hypothesis: A New Model for Hippocampal Neurogenesis

G. Enikolopov, J. Encinas, A. Koulakov

New neurons are continuously generated throughout the life of an animal in at least two areas of the mammalian brain: olfactory bulb and hippocampal dentate gyrus. Hippocampal neurogenesis dynamically responds to a multitude of extrinsic stimuli and may be important for behavior, pathophysiology, brain repair, and the response to drugs that modulate mood. New neurons are produced from a limited population of stem cells whose number declines with age. What factors determine the rate of decline in the hippocampal stem cell population? How can the rate of production of new neurons be varied? In this study, we have shown that soon after a stem cell is used for production of new neurons, it becomes an astrocyte, i.e., it leaves the pool of neuroprogenitors. Therefore, one can think of the stem cells as disposable: Once they are used, they cannot be reused. The decline in the number of stem cells therefore occurs in a use-dependent manner. Does it mean that the more neurons are produced, the faster the stem cells are lost? Not necessarily. The sequence of transformations that a cell must undergo before if becomes a new neuron is very complex. One of the steps involves an intermediate form of neuroprogenitors that are called amplifying cells. These cells divide rapidly, about once per day, to make more neurons. It turns out that antidepressant drugs, such as Prozac, affect the divisions of the intermediate amplifying cells without much impact on primary (disposable) stem cells. Together with the experimental group of Grisha Enikolopov at CSHL, we have developed a computational model for the division/differentiation cascade that leads to the production of new neurons. Comparing the computational model to experimental results leads to detailed information about the changes occuring in the neurogenesis cascade due to antidepressant drugs, aging, and other therapies. Our approach will provide unique insight into the computational properties of the neural stem cells and their importance for mental health.

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THE NEURAL ORGANIZATION OF OLFACTORY BEHAVIOR

Z.F. Mainen G. Felsen S. Ranade
A. Kepecs M. Smear
M. Murakami

Frontal Control of Impulsive Action

M. Murakami

Inhibition of behavior is as important as its generation, and impulsivity is a common central feature of pathologies including attention deficit hyperactivity disorder, drug addiction, and obsessive compulsive disorder. Consider the situation in which you wait for a bus but it is delayed. You have no idea when it will arrive. Another option is to take a cab, which costs much more but is available immediately. How long do you wait for the bus? When do you give up waiting for the bus and decide to take the cab? In such circumstances, your brain must compute the costs and benefits of the choices and use that information to choose an action—waiting or giving up. In this study, we are investigating the role of medial prefrontal cortex (mPFC) and secondary motor cortex (M2) in deciding whether to wait or not.

The aim of the project is to understand how the frontal cortex is involved in inhibiting impulsive actions. The specific aims are to (1) reveal the activity of frontal cortical neurons while rats are engaged in impulse control task and (2) examine the effect of inactivating subregions of the frontal cortex on impulse control behavior.

In our task, a rat is faced with a waiting problem similar to the "bus or cab" dilemma. There are two nose poke ports; one is for waiting and the other is for delivering water reward. A short delay (400 msec) after a rat pokes in the waiting port, a first tone is played, and this signals the availability of a small reward at the reward port. If the rat goes to a reward port after the first tone, it receives a small amount of water reward. If the rat waits longer in the waiting port, instead of going to the reward port, then a second tone is played after a random delay period of up to several seconds. If the rat goes to the reward port after this second tone, it receives a larger amount of water reward. In this task, after the first tone is played, the rat is confronted with a choice between going to the reward port to get a small reward or waiting for a second tone to get a large reward.

In 2008, we recorded from neurons in the mPFC and the secondary M2 while rats were performing the impulse control task. We found that the activity of subpopulations of mPFC and M2 neurons predict the impulse control performance of rats in the task, which suggests a role for those frontal areas in withholding impulsive action.

To examine the causal role of the frontal cortex in withholding impulsive action, we examined the effect of reversible inactivation of the mPFC on impulse control behavior. Inactivation of the mPFC impairs the ability of rats to inhibit impulsive action. These results suggest a role of the frontal cortex in withholding impulsive actions.

Behavioral results showed that the time a rat is willing to wait for a given set of reward amounts and delays varies randomly from trial to trial. In some trials, the rat will respond immediately after the first tone; in others, it will withhold responding until after the second tone, and still in others, it will wait well past the first tone, but give up before the second.

Motor Planning in the Rat Superior Colliculus

G. Felsen

The superior colliculus (SC) has an important role in spatial orienting across many species. We recently recorded SC activity in freely moving rats performing a spatial choice task. In this task, an odor cue delivered at a central port determines whether water will be delivered upon entry into the left or right reward port. As soon as the rat decides on the identity of the odor, it exits the odor port, orients left or right, and enters the selected reward port. We found that the activity of single SC neurons during the 100 msec preceding odor port exit is predictive of the rat's choice (left vs. right) and that this activity is necessary for normal movement production.

We now ask whether this activity is related to the initiation of movement or to some aspect of planning the movement (e.g., selecting the direction of movement). Conceivably, the movement could be planned in a separate region (or regions) upstream, and the SC would simply be responsible for initiating the planned movement. Alternatively, the SC could be involved in the planning itself. We addressed this issue in two ways. First, we reasoned that if the SC were involved only in movement initiation, its activity should be independent of the stimulus. We therefore asked whether some feature of the stimulus

was encoded in the SC activity, in addition to the movement direction. We found that preceding the movement initiation, a subset of cells encoded the identity of the odor in addition to the upcoming movement, potentially linking the stimulus and the action. This suggests that the SC is not simply responsible for initiating movement.

Next, we recorded SC activity in a new set of rats performing a delayed-response version of the spatial choice task. In each trial of this task, the rat was required to remain in the odor port for 500 or 1000 msec following odor presentation before initiating its movement toward the water port. Presumably, the rat still selects the direction of movement within a few hundred milliseconds, as in the reaction-time task. Thus, this task allows us to dissociate the time corresponding to the decision from the time of movement initiation. We found that the activity of many choice-predictive cells peaked soon after odor presentation began and remained elevated until the rat exited the odor port. The timing of this activity is more consistent with a role for the SC in selecting or planning movements, rather than simply initiating them. Thus, the SC may be a critical component of the circuit for movement planning.

Diversity and Precision of Neural Activity in Serotonergic Brain Stem Nuclei

S. Ranade

Serotonin is a neurotransmitter implicated in a diverse range of physiological functions and behaviors as well as psychiatric disorders including depression and anxiety. Serotonin is released by neurons in a set of brain stem nuclei called the raphe nuclei. The raphe system is by far the most complex divergent neuromodulatory system in the brain. Our current understanding of serotonin function has been gained mostly from pharmacology and lesion studies. Neuronal recordings from animals performing well-controlled behavioral tasks have greatly increased our knowledge of dopamine and norepinephrine function, but there have been few recordings from raphe neurons during behavioral tasks. We hypothesize that such studies will yield novel insights about raphe function, particularly at fast timescales.

In this project, we recorded from raphe nuclei in rats performing a two-alternative choice odor discrimination task (as described above). Rats were trained to associate individual odors with water reward at one of two choice ports. Correct responses were rewarded probabilistically after a variable delay. This paradigm allowed us to study sensory, motor, and reward-related responses with high temporal precision by aligning to nose poke events.

Raphe neurons showed diverse firing properties with respect to waveform characteristics, firing rate, and sleep state modulation. Neuronal responses were analyzed with respect to four epochs: odor sampling, movement, reward anticipation, and reward consumption. Firing rates of >70% neurons were modulated during at least one behavioral epoch, with a large fraction tuned to multiple events. Many neurons responded to behavioral events within 100 msec, whereas some were even more precisely time-locked (order 20 msec), showing a very strong (>40 sp/s) phasic response, apparently to the click produced by the water valve opening.

A wide range of event-tuning characteristics were observed in the recorded population. During odor sampling, approximately one-third of units showed a decrease in firing rate. A subset of neurons also showed odor-induced activation that, in rare cases, was stimulus selective. During the movement epoch, an equal proportion of neurons showed enhancement and suppression of firing. There were few instances of direction-selective tuning. A large proportion of neurons (~40%) were inhibited during reward anticipation. A small subset of neurons (10%) showed changes in firing rate around the time of expected reward. Putative serotonin neurons showed no obvious association with a specific response profile.

These recordings demonstrate that raphe neurons are rapidly and precisely modulated by diverse behavioral events. This is in accord with the known diversity of serotonin function and the difficulty in accounting for it with a simple unified theory. Functional diversity of raphe responses likely reflects in part the diversity of intrinsic properties and synaptic connectivity of neurons within the nucleus. Classification of units into putative serotonin and nonserotonin neurons did not yield any obvious simplification of response diversity, highlighting the need for methods relating firing patterns to precise identification of neuronal cell types. Finally, the observed functional diversity of raphe neurons is consistent with the possibility that significant information processing may occur within the raphe itself. Future studies will focus on developing novel molecular genetic approaches to selectively label and record activity of populations of raphe neurons that are biochemically homogeneous and/or share similar connectivity patterns.

Studying the “Where” Problem in Mammalian Olfaction

M. Smear

Localizing the source of an odor often matters as much as identifying it: Distinguishing a meal from a predator

is futile if you avoid the former or approach the latter. Although our understanding of odor identification—the "what" problem of olfaction—has progressed considerably, the "where" problem of olfaction has received relatively little attention. We know that rats excel at odor-guided navigation, but we do not know what behavioral strategies and neural circuits they use. A critical challenge to elucidating the neural mechanisms underlying odor tracking is reducing the behavior to a form suitable to psychophysical techniques. To this end, we have designed an "olfactorium," which is essentially a two-armed maze in which the rat has to navigate to the correct arm based on concentration cues. Using a photoionization detector, we can obtain a statistical characterization of odor-concentration patterns with high spatial, temporal, and concentration resolution. In addition, we can precisely track the nose position of a freely moving rat at video-rate time resolution in real time. By combining odor-concentration measurements with tracked trajectories, we hope to reconstruct the time-varying odor-concentration pattern experienced by a navigating rat and thereby reverse-engineer its navigation strategies. In the future, we intend to exploit rodent neuroscience's growing arsenal of tools for electrophysiology and transgenesis in the context of olfactospatial psychophysics to understand the neural circuitry of odor source localization.

The Neural Representation and Impact of Decision Confidence

A. Kepecs, H. Zariwala, N. Uchida

If you are asked to evaluate your confidence in something you know—how sure are you that it is true—you can readily answer. What is the neural basis for such judgments? Is knowledge about beliefs an example of the human brain's capacity for self-awareness? Or is there a simpler explanation that might suggest a more basic yet fundamental role for uncertainty in neural computation?

We addressed these questions by experimentally manipulating the difficulty of perceptual decisions and searched for a neural correlate of rats' confidence about getting a reward. We trained rats in a two-choice odor-mixture categorization task that is well suited for studying decision-making in rodents. In this task, rats report the dominant odor-mixture component by entering different choice ports. Their performance is nearly perfect for pure odors and drops systematically with mixture ratio (difficulty). Using this task, we could systematically manipulate the uncertainty of individual discriminations by varying odor-mixture ratio. We found a population of neurons in the orbitofrontal cortex (OFC) whose firing tracked the difficulty of the decision and, surprisingly, could even anticipate the outcomes of different trials of the same difficulty. We were able to concisely explain this pattern of data using a simple model of decision confidence. We then devised a behavior that showed that rats could actually report their confidence when probed with a subsequent decision. This demonstrated that rats, like humans, sometimes know how certain they are in their answers. Taken together, our results support the idea that decision confidence may be a widespread and fundamental element of neural processing contributing to adaptive behavior.

We began to extend these observations by testing whether confidence-related neural activity in the OFC is causally related to confidence judgments. Specifically, we began to test whether the OFC has a primary role in confidence-guided behaviors by inactivating it. We hypothesize that shutting off the OFC leaves the odor-guided behavior intact while impairing the use of confidence information to guide leaving decisions. In collaboration with Erin Romberg (2007 Undergraduate Research Program participant), we are testing this hypothesis. Our preliminary results suggest that lesions of the OFC indeed significantly impair rats' ability to report confidence by waiting for delayed rewards without affecting olfactory discrimination performance. Notably, these findings are consistent with studies of human patients with orbitofrontal lesions who are unable to judge the degree of uncertainty, indicating that the functions of the OFC that we are investigating in rats are conserved across species.

These results suggest that a dynamic representation of and access to an internal measure of decision confidence is fundamental to adaptive behavior—even in rats—and provide insights into the nature of the neural processes underlying decision-making. Our findings establish neuronal correlates and behavioral assays that lay a foundation for a broader examination of the neural mechanisms underlying confidence estimation.

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NEURAL CODING IN THE *DROSOPHILA* OLFACTORY SYSTEM

G. Turner R. Campbell E. Demir T. Hige K. Honegger E. Gruntman

How does the brain process sensory information to produce a behavioral output? How does experience modify those internal representations? We address these questions in the appealingly simple olfactory system of the fruit fly. This is an extremely powerful model system because it allows us to combine genetic manipulations of neural activity with electrophysiological and imaging-based measures of neural activity. Our overall goal is to understand how olfactory information is represented in terms of neural spike trains and how associative learning modifies these representations.

There are three primary research directions in the lab. The first is to understand how the olfactory system is able to create distinct perceptions of the huge number of different odors in the environment. How the brain creates these distinct perceptions is the basis of how the accuracy of memory formation and recall is achieved. The second question is how olfactory cues can be used to localize an odor source. Insect disease vectors typically locate their hosts by smell, but relatively little is known about how insects follow rapidly varying plumes of odor to their source. The third avenue is to investigate how olfactory associations are formed during learning.

COMBINATORIAL CODING IN THE OLFACTORY SYSTEM

Distinguishing different odors presents a particularly challenging problem for the nervous system because odors are so chemically diverse. To detect such a variety of compounds, species have evolved a large array of olfactory receptors. In mammals, there are ~1000 of these olfactory receptors, whereas in *Drosophila*, an appealingly simpler model system, there are only 62. It is thought that olfactory information is encoded combinatorially and that combining information across sensory channels expands the range of possible olfactory percepts beyond the raw number of sensory channels.

For combinatorial coding to be an effective strategy, there must be neurons at deeper layers in the brain that respond selectively to specific combinations of input activity. Highly selective neurons that carry sparse stimulus representations are a general feature of brain areas involved in learning and memory, including hippocampus and cerebellum, and considerable theoretical work predicts that sparse codes are optimized for memory storage. We are testing the hypothesis that it is the neurons in the learning and memory center of the *Drosophila* brain, the mushroom body (MB), that respond selectively to particular combinations of input activity.

To determine whether different sensory channels converge on individual MB neurons, we are stimulating specific neurons at the upstream layer of the olfactory pathway and recording responses in MB neurons. To do this, we use an approach that allows us to activate neurons with light. We use genetic techniques to express an ion channel that activates neurons when illuminated with blue light. We have built a customized light delivery system that allows us to deliver blue light in precisely timed flashes, and we found that we can control electrical activity very accurately in *Drosophila* neurons. Using this approach to stimulate precisely defined sets of neurons at an early stage of the olfactory pathway, we are determining the number of different inputs an individual MB neuron integrates (i.e., how combinatorial is the olfactory code). Because we have such precise control over neural activity with this method, we are also asking what is the minimal amount of activity that the animal can detect. This is a very basic parameter for understanding neural coding in any system: Is one spike interpretable by the deeper layers of the brain or is it within the operating noise of the system, so perhaps only 10 or 100 spikes are significant. Understanding (1) how many different inputs there are to a given MB neuron and (2) what are the relevant strengths of those inputs will reveal two of the most important aspects underlying the combinatorial code and how it is integrated to generate highly selective neuronal responses that are a hallmark of learning and memory centers in the brain.

ODOR SOURCE LOCALIZATION

Insects typically locate mates and prey using olfactory cues; understanding how they achieve this could therefore open up new strategies for controlling insects that act as disease vectors. We are using a combination of behavioral experiments and electrophysiological recordings to investigate the neural mechanisms of odor local-

ization. To study odor-driven locomotion, we have developed an apparatus to precisely measure the movements of a fly under conditions where we can precisely control the odor stimuli it encounters. The fly is tethered via its thorax to a small wire, and its legs make contact with a tiny ball that floats frictionless on a stream of air. The leg movements of the fly translate into rotational movements of the ball, which we can use to reconstruct the overall walking trajectory of the fly as we present various types of olfactory stimuli: (1) an odor stream from one side of the animal, (2) rising/falling odor gradients, and (3) rapid pulses with increasing/decreasing interpulse intervals. We have shown that flies have a directional response to odor in this behavioral apparatus, and we are testing the relevant cues that drive this behavior.

We are investigating the neural mechanisms underlying odor localization by recording neural activity while presenting flies with a rapidly fluctuating odor stream. Preliminary experiments suggest that MB neurons very rapidly adapt to such fluctuating odor stimuli. Thus, MB neurons may accurately represent odor identity, but changes in odor concentration may be encoded in other layers of the olfactory pathway. We plan to explore other layers of the olfactory system to determine whether and how they represent changes in odor concentration. We are also very interested in investigating the mechanisms that enable MB neurons to create concentration-invariant representations, because these are likely to be very useful for olfactory memory—in our memory, the smell of an apple is an apple over a wide range of intensities.

OLFACTORY LEARNING AND MEMORY

Our goal here is to identify the changes in neural activity that occur during learning and to understand the basic mechanisms underlying those changes. We have been examining the role that neuromodulatory pathways, such as dopamine, have in learning and memory. We find that one particular neuromodulator increases the activity of neurons that send olfactory information to the fly's learning and memory center. This increase in activity could have multiple effects on learning and memory. One possibility is that it causes stronger neural responses to olfactory stimuli, which could facilitate olfactory learning. Alternatively, it could have a direct effect on the strength of the synaptic connections that the olfactory neurons make in the learning and memory center. We are testing these possibilities, with a focus on linking neuromodulator effects with the general process of synaptic plasticity.

We are also directly examining how synaptic connections among neurons can be strengthened or weakened by neural activity in the fly brain. Using the light-activated ion channel mentioned above, we can precisely control the activity of neurons that send input to the MB. Our preliminary data suggest that when we activate these inputs with a particular timing, the input synapses strengthen. This could enable neurons that initially respond only to a taste to begin to respond to an odor once synapses of olfactory inputs are strengthened. These observations are preliminary, but they are important because they give us a sense of how learning could take place.

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NEURAL CIRCUITRY OF UNDERLYING NORMAL AND ABNORMAL PROCESSING IN THE CORTEX

A. Zador K. Borges, T. Hromadka, S. Jaramillo, G. Otazu, H. Oviedo, H. Oyibo, A. Reid, Q. Xiong, Y. Yang, P. Znamenskiy

My laboratory is interested in how neural circuits underlie normal processing and attention in the auditory cortex and how this processing is disrupted in cognitive disorders such as autism. To address these questions, we use a combination of computational, electrophysiological, and imaging techniques at the molecular, synaptic, cellular, circuit, and behavioral levels.

Disruption of Auditory Cortical Circuits by Autism Candidate Genes

T. Hromadka, Q. Xiong

Autism is a highly heritable disorder thought to arise through disruption of neural circuits. Many candidate genes have been implicated, but how these genes lead to the autistic phenotype remains unclear. We hypothesize that the circuit defect underlying autism involves an imbalance between excitatory and inhibitory neural activity. To test this hypothesis, we are using in vitro and in vivo methods to assess circuit dysfunction in the auditory cortex. Previous work from my laboratory has shown that excitation and inhibition are exquisitely balanced in the auditory cortex, and so this assay should be a very sensitive measure of disruption in animal models in which autism candidate genes have been disrupted.

Effect of Engaging in an Auditory Task on Responses in the Auditory Cortex

G. Otazu, Y. Yang, L. Tai

Although systems involved in attentional selection have been studied extensively, much less is known about nonselective systems. To study these preparatory mechanisms, we have compared activity in the auditory cortex elicited by sounds while rats performed an auditory task ("engaged") with those elicited by identical stimuli while subjects were awake but not performing a task ("passive"). Surprisingly, we found that engagement suppressed responses, an effect opposite in sign to that elicited by selective attention. In the auditory thalamus, engagement enhanced spontaneous firing rates but did not affect evoked responses. These results demonstrate that in auditory cortex, neural activity cannot be viewed simply as a limited resource allocated in greater measure as the state of the animal passes from somnolent to passively listening to engaged and attentive, but that instead, the engaged condition possesses a characteristic and distinct neural signature in which sound-evoked responses are paradoxically suppressed (Otazu et al., in press).

Role of Interneurons in Auditory Cortex Function

K. Borges, A. Reid

Fast synaptic inputs to neurons in the auditory cortex are either inhibitory or excitatory. Cortical interneurons are tremendously diverse. One interneuron subclass, defined molecularly by the expression of parvalbumin ("PV+"), seems ideally positioned to mediate the fast component of the characteristic barrage of inhibition elicited by a sound. We are testing the hypothesis that PV+ inhibitory interneurons mediate fast sound-evoked inhibitory synaptic currents in the auditory cortex.

Our proposal seeks to establish a causal link between a physiological property—the fast sound-evoked inhibition that contributes to receptive field dynamics—and a component of the underlying cortical circuitry. We approach the problem at three different levels, from brain slices through in vivo physiology to behavior. Although we are currently focusing on the role of one particular interneuron subclass (PV+), our approach combining electrophysiological and molecular tools can readily be generalized to other subclasses and can be extended to probe the circuitry underlying other sensory- and behaviorally elicited neuronal responses.

Temporal Expectation Modulates Neuronal Responses in the Auditory Cortex

S. Jaramillo

When a stimulus occurs at a predictable instant in time, anticipation of the stimulus improves the speed and accuracy with which it is detected. We have developed a two-alternative-choice task paradigm in freely moving rats to study the neural mechanisms underlying this phenomenon in the auditory system. Behavioral measurements confirm that valid expectations improved both reaction times and detection thresholds. We are also using tetrodes to record responses from single neurons in the auditory cortex of rats performing the task. Responsive neurons often showed an increase in evoked response to tones immediately preceding the expected moment of appearance of the target, when compared to responses to the same tones occurring long before the expected target. In addition, grouping behavioral trials according to the subject's reaction time reveals correlations between the strength of the neuronal responses and performance.

Identifying Neurons with Channelrhodopsin-2 during In Vivo Recording

S. Lima, T. Hromadka

Neural circuits consist of a heterogeneous mixture of neurons with different neuroanatomical projections and patterns of molecular expression. Recordings of neural activity in behaving animals reveal tremendous functional heterogeneity as well: Nearby neurons often respond very differently to the same stimulus or action. However, little is known about how this structural circuit-level heterogeneity contributes to function, in part because of the technical difficulty of identifying neurons during in vivo recordings in behaving animals.

To overcome this difficulty, we have developed a technique that allows us to "tag" subpopulations of neurons for identification during in vivo electrophysiological recordings. The tag is a light-gated ion channel—the algal protein channelrhodopsin-2 (ChR2)—whose expression can be genetically restricted to a subpopulation of neurons. In the subpopulation of neurons expressing ChR2, a brief flash of blue light triggers a single action potential with millisecond precision.

We are using this approach to test the hypothesis that neuroanatomical connectivity represents one important structural correlate of the functional diversity in the rodent cortex. To do this, we restrict ChR2 expression to subsets of neurons in the rat auditory cortex (ACx). ACx pyramidal neurons project to multiple brain regions, including the amygdala, the posterior parietal cortex, or the contralateral ACx, and presumably carry different information about auditory stimuli to these centers. To target ACx neurons specifically based on their projection pattern, we inserted the ChR2-coding region into a herpes simplex virus (HSV). The HSV travels in a retrograde fashion through the axons of infected neurons. ChR2-tagged neurons, i.e., neurons projecting to the infected area, can be identified by their low latency and reliable spiking response to a brief light flash. Thus, for example, we have used this approach to identify the subpopulation of layer-5 ACx neurons that project to the contralateral cortex.

This approach is general, in that any population to which expression of ChR2 can be genetically restricted can be tagged. Promising future applications include tagging of different subpopulations of neurons based on promoters (e.g., for subclasses of inhibitory interneurons) and tagging of neurons in different cortical layers.

Mapping of Auditory Cortex Circuitry Using Laser-scanning Photostimulation

H. Oviedo [in collaboration with I. Bureau and K. Svoboda, Cold Spring Harbor Laboratory]

It is widely assumed that the organization of the sensory cortex can be described by a "canonical" circuit. According to this view, sensory input from the thalamus arrives at cortical layer 4, propagates to layer 2/3, and then descends to layer 5 before exiting a brain region. However, until recently, it has been technically difficult to test this hypothesis directly. We are applying a new approach—laser-scanning photostimulation—to map the circuitry within the rodent auditory cortex. Using this approach, we can directly compare the circuitry within the auditory cortex with that of other sensory cortices, such as the better-studied barrel cortex. Preliminary results indicate that although the auditory cortex is organized according to many of the same general principles, the detailed structure appears to be quite different.

Using Cortical Timing Information to Guide Behavior

Y. Yang, G. Otazu, M. DeWeese

It is well established that animals can exploit the fine temporal structure of some stimuli; for example, inter-

aural time differences of less than 1 msec are used for spatial localization of sound. It is also clear that cortical neurons can lock with millisecond precision to the fine temporal structure of some stimuli. However, it has been difficult to establish whether the fine temporal structure of cortical responses can be used in a behavioral context to guide decisions. Indeed, in the case of spatial localization of sound, the relevant interaural time difference cues are processed below the level of the cortex by means of specialized circuitry.

We have therefore adopted a direct approach to probe the precision with which cortical timing information can be used to guide behavior in the rat (Yang et al. 2008). To bypass subcortical auditory pathways, we stimulate the primary auditory cortex directly, using transient biphasic current trains delivered via chronically implanted intracortical microelectrodes. The behavioral paradigm we use is a two-alternative-choice task in which stimulus 1 consists of the simultaneous stimulation of two intracortical sites (A and B) and stimulus 2 consists of sequential stimulation of the two sites separated by a brief interval dT (A–dT–B). After the subjects are trained to perform to criterion (1–2 weeks) with the initial long interstimulus interval (dT > 50 msec) for stimulus 2, we probe the subjects' psychophysical threshold by reducing dT, until the subjects can no longer distinguish between the two stimuli. Our experiments indicate that the cortex can make use of information on a timescale as short as 3 msec (Yang et al. 2008).

Sparse Representation of Sounds in the Auditory Cortex

T. Hromadka

How do neuronal populations in the auditory cortex represent acoustic stimuli? Although sound-evoked neural responses in the anesthetized auditory cortex are mainly transient, recent experiments in the unanesthetized preparation have emphasized subpopulations with other response properties. To quantify the relative contributions of these different subpopulations in the awake preparation, we have estimated the representation of sounds across the neuronal population using a representative ensemble of stimuli. We used a cell-attached recording with a glass electrode, a method for which single-unit isolation does not depend on neuronal activity, to quantify the fraction of neurons engaged by acoustic stimuli (tones, frequency-modulated sweeps, white noise bursts, and natural stimuli) in the primary auditory cortex of awake head-fixed rats. We find that the population response is sparse, with stimuli typically eliciting high firing rates (>20 spikes/sec) in less than 5% of the neurons at any instant. Some neurons had very low spontaneous firing rates (<0.01 spikes/sec). At the other extreme, some neurons had driven rates in excess of 50 spikes/sec. Interestingly, the overall population response was well described by a lognormal distribution, rather than the exponential distribution that is often reported. These results represent the first quantitative evidence for sparse representations of sounds in the unanesthetized auditory cortex. Our results are compatible with a model in which most neurons are silent much of the time and in which representations are composed of small dynamic subsets of highly active neurons (Hromadka et al. 2008; Koulakov et al. 2009).

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong J. Beshel M. Pagani
H.-C. Chiang L. Wang
I. Hakker C. Xu

We combine genetic and functional analyses in the study of the neuronal and molecular bases of learning and memory in *Drosophila*. We are involved in three projects to identify new molecular and neuronal events that are essential in learning and memory. One project is related to the normal and pathological function of the gene *corkscrew* that is associated with Noonan syndrome (NS). This syndrome shows a number of developmental defects including mental retardation. We are interested in how point mutations associated with NS affect the so-called "spacing effect" in memory formation. In the second project, we are studying the roles of the Aβ42 peptide associated with Alzheimer's disease in the modulation of synaptic plasticity and memory loss in *Drosophila*. The third project is related to the integration of sensory information in the mushroom body (MB), a higher olfactory center that is essential for olfactory associative learning. In this project, we are examining the functional imaging of airflow response in the *Drosophila* MB.

Corkscrew Activity in *Drosophila* Regulates the Intertraining Interval Needed for Long-term Memory Formation

M. Paganil

NS is a genetic disorder comprising a range of developmental and cognitive abnormalities. One cause is gain-of-function (GOF) mutations in *PTPN11*, which encodes the tyrosine phosphatase SHP-2. To gain insights into these cognitive alterations, we used transgenic *Drosophila* NS models inducibly expressing the fly SHP-2 ortholog, corkscrew (CSW). Our studies revealed that GOF CSW impairs long-term memory (LTM) formation even when first expressed in adulthood. A universal property of LTM induction is the requirement for repeated training sessions spaced over time. The molecular basis of this spacing effect is completely unknown. We demonstrated that wild-type CSW overexpression dramatically shortened the interval between training sessions required for LTM induction. Conversely, the LTM formation defect in the NS fly models could be rescued by increasing the interval between trials. Thus, we provide the first evidence that the spacing effect has a molecular basis and that CSW/SHP-2 is key in its regulation. This project is a collaboration with Drs. Oishi and Gelb at Mount Sinai.

PI3 Kinase Modulates Aβ Peptide Aggregation and Memory Loss in a *Drosophila* Model of Alzheimer's Disease

H.-C. Chiang, L. Wang, I. Hakker

Expression of a secretary form of β-amyloid peptide 42 (Aβ42) in the *Drosophila* brain recapitulates major features of Alzheimer's disease (AD), including age-dependent memory loss, neurodegeneration, and accumulation of Aβ fibril deposits. To gain insight into the molecular basis underlying memory loss, we initiated our study by examining synaptic plasticity, which shows that long-term depression is enhanced by expression of Aβ42 and that such enhancement is linked to increased phosphoinositol-3 kinase (PI3K) activity. To confirm this electrophysiological finding, we directly assessed insulin-stimulated PI3K activity, which revealed an elevated basal level of PI3K activity in Aβ42-expressing neurons and unresponsiveness of these neurons to further insulin stimulation. Because AD brain tissues are known to be insulin-resistant, this observation led us to test the behavioral effects of such molecular alteration. Genetic silencing or pharmacological inhibition of PI3K activity significantly rescues age-dependent memory loss induced by expression of Aβ42. This conclusion is further supported by genetically manipulating the activity of dPTEN, a phosphatase-antagonizing PI3K activity. Further investigation indicates that accumulation of Aβ42 oligomers (dimmer and trimmer) and Aβ42 fibril deposits is significantly reduced. Thus, this study suggests that the PI3K pathway has a critical role in mediating the Aβ42 toxic effects on synaptic plasticity and on memory loss in association with modifying Aβ42 aggregation, but it has no effects on Aβ42-induced neurodegeneration.

Neural Representations of Airflow in the *Drosophila* Mushroom Body

A. Mamiya, J. Beshel, C. Xu

The *Drosophila* MB is a higher olfactory center where olfactory and other sensory information are thought to be associated. However, how MB neurons of *Drosophila* respond to sensory stimuli other than odor is not known. Here, we characterized the responses of MB neurons to a change in airflow, a stimulus associated with odor perception. In vivo calcium imaging from MB neurons revealed surprisingly strong and dynamic responses to an airflow stimulus. This response was dependent on the movement of the third antennal segment, suggesting that Johnston's organ may be detecting the airflow. The calyx, the input region of the MB, responded homogeneously to airflow on. However, in the output lobes of the MB, different types of MB neurons responded with different patterns of activity to airflow on and off. Furthermore, detailed spatial analysis of the responses revealed that even within a lobe that is composed of a single type of MB neuron, there are subdivisions that respond differently to airflow on and off. These subdivisions within a single lobe were organized in a stereotypic manner across flies. For the first time, we could show that changes in airflow affect MB neurons significantly and that these effects are spatially organized into divisions smaller than previously defined MB neuron types.

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Mario Pagani

BIOINFORMATICS AND GENOMICS

Bioinformaticians in literally dozens of labs are devising new capabilities that their colleagues are putting to novel uses in their experiments. These technologies, it is universally acknowledged, are prerequisites for satisfactory progress in diverse areas of research, from cancer to neurological diseases such as Alzheimer's and Parkinson's to neuropsychiatric illnesses such as schizophrenia and autism to all aspects of plant biology and genetics. At the Lita Annenberg Hazen Genome Sequencing Center, directed by W. Richard McCombie, CSHL scientists have been among the leaders in devising, refining, and updating techniques that enabled the sequencing of human and other "model" genomes earlier in this decade. They are currently generating the technical and conceptual means for labs worldwide to use a new generation of sequencers that are paving the way to low-cost individual genomes and an era of personalized medicine.

The insights of W. Richard McCombie and colleagues have led to the introduction and optimization of novel methods of high-throughput genome sequencing. His team has made it possible to catalog variation among individual organisms in a way that would have been unthinkable at the beginning of the decade. Having brought online a new generation of Solexa sequencers at CSHL this past year, McCombie's team optimized their function to a level at which 10 billion DNA bases (single "rungs" on the double-helical DNA "ladder") can be sequenced in a typical day, and on some days as many as 20 billion. McCombie has been a leader of efforts to sequence the flowering plant *Arabidopsis thaliana*, the fission yeast *Schizosaccharomyces pombe,* and *Homo sapiens*. New projects are under way to sequence genes of special interest, including *DISC1*, a strong candidate gene for schizophrenia, and genomic regions likely implicated in bipolar illness. With the Memorial Sloan-Kettering Cancer Center, they are using a method called hybrid resequencing, developed with Greg Hannon here at CSHL, to look at mutations in samples collected from patients with prostate cancer.

Jonathan Sebat's laboratory is studying the role of genetic variation, and particularly gene copy-number variation, in schizophrenia, autism, and other neuropsychiatric illnesses. This past year, Sebat and collaborators discovered that a significantly increased rate of rare structural mutations exists in the genomes of individuals with schizophrenia as compared with healthy controls. They found, moreover, that the mutations were powerful, increasing individual risk 10–20 times, and that in people with schizophrenia, the genes disrupted by the mutations nearly half the time were involved in pathways known to be important in brain development. One implication is that the universe of genetic risk factors for schizophrenia consists of many different rare mutations, each one present in comparatively few individuals or even a single one. Thus, the paradox: Despite the large constellation of rare mutations contributing to the disease, one must sift through thousands of cases before any one of them is likely to appear more than once. In prior work, Sebat, in collaboration with Michael Wigler at CSHL, discovered that spontaneous mutations—genetic errors in children that do not occur in either parent—are far more common in autism than previously thought.

Lincoln Stein's lab is developing databases, data analysis tools, and user interfaces to organize, manage, and visualize the vast body of information being generated by biologists. One of the unsolved mysteries of the genome is how its genes are precisely regulated to promote orderly growth and development and respond to changes in the environment. The modENCODE project (*mod*el organism *enc*yclopedia *o*f *D*NA *e*lements) is an international consortium organized to find and characterize the elements that regulate the genomes of the fruit fly and the roundworm. Last year, the Stein lab established the modENCODE *D*ata Coordination Center, which is responsible for collecting, integrating, and publishing the information collected by the consortium in a form that can be extended and combined with information from other human and model organism genome databases.

The HapMap project is another focus of Stein's lab, a database of human single-base-pair variations. Last year, the HapMap database was expanded from information on the genetic variability of 4 populations to 11 human populations, including individuals from Africa, Asia, Europe, Mexico, and the Indian

subcontinent. The variability information is integrated with recently published information on more than 50 susceptibility regions in 20 common human diseases, including diabetes, rheumatoid arthritis, Crohn's disease, coronary artery disease, and bipolar disorder.

The Stein lab also manages and curates the WormBase and Reactome databases. WormBase, a database of the genome and biology of the roundworm *Caenorhabditis elegans,* gives users quick access to the large *C. elegans* literature. It has been integrated by Stein's group with an open-source online publication called WormBook, which now has 140 monograph "chapters" that link to gene entry data in WormBase. Reactome, an interactive database of fundamental biological pathways in humans, integrates the peer-reviewed literature with high-throughput genomic information, including a growing database of protein–protein interactions (now covering more than half of the annotated genome). Finally, Stein's group has begun work on the iPlant Collaborative, an effort to better enable plant biology researchers to collaborate in cyberspace.

Using multidisciplinary approaches that combine computational analysis, modeling, and prediction with experimental verification, Doreen Ware's lab seeks a deeper understanding of genome organization in plants. By looking comparatively across the genomes of plants in the same lineage, Ware and colleagues seek answers to such questions as, How are genes conserved and lost over time? What are the fates of duplicated genes? Her team also studies gene regulation in plants, specifically looking at *cis*-regulatory elements and characterizing microRNA genes and their targets. This past year, the lab has been responsible for providing annotations of the maize genome for an international consortium. Within their own lab, they have also brought new, fully sequenced genomes into an existing integrated data framework, to enhance the power of their comparative studies. This framework now encompasses the genomes of *Arabidopsis*, maize, rice, sorghum, grape, and poplar. They have devoted special attention to examining diversity within species, particularly maize and grape, in assays that make use of powerful new genotyping technology.

DEVELOPMENT OF NEXT-GENERATION SEQUENCING TECHNOLOGY

W.R. McCombie J. Busuttil, Y. Cai, L. Cardone, M. Chen, E. Ghiban, M. Kramer, D. Lewis, S. Mavruk, S. Muller, J. Parla, S. Pierce, D. Rebolini, L. Spiegel

EXPANDING OUR ABILITY TO EXAMINE GENOME VARIATION

The genomic analysis we are carrying out is a dramatic departure from the genomics of pre-2007. It uses all new instruments, lab operations, and software for analyses. To do this analysis, we had to build substantial infrastructure in the areas of lab tools and procedures, human resources, and computational resources. Now in place, these capabilities are beginning to radically change the way in which we can find variation in genome structure that might be associated with phenotypic change such as human disease.

Building Infrastructure

L. Cardone, D. Rebolini, S. Mavruk, M. Kramer, D. Lewis

The major lab resource that we needed to put in place was "next-generation sequencing" capability. These instruments became generally available in the late winter to spring of 2007, but we were able to obtain an early test version in December 2006. We had the instrument in production use by late January–early February 2007, making us one of the first sites in the world to develop this capability. We have upgraded these instruments and merged them in the same physical space with instruments obtained from other funding sources to achieve a considerable economy of scale.

To perform the next-generation sequencing on a large scale, we hired a number of staff including technicians and postdoctoral fellows and trained them in the necessary skills. The technicians underwent extensive training as they learned to use the new sequencing instruments as well as to diagnose problems as they arose. The postdoctoral fellows have developed new ways to carry out the isolation of regions of the genome of interest from patient samples and developed advanced methods of data analysis.

The development of the appropriate computational analysis for next-generation sequencing was a substantial challenge: Network capabilities needed to be upgraded, software was installed and made operational on large compute clusters, and large, high-speed storage arrays were tested and placed into use. All of these steps were performed and are now in place. As a result, we have world-class sequencing capabilities and are applying them primarily to psychiatric genetics.

DEVELOPMENT OF CAPABILITIES

We began with a single sequencing instrument in January 2007. At that time, we were learning how to operate the instrument, prepare samples, analyze data, and troubleshoot. Initial efforts with the instruments produced mixed results. However, as we worked through the various issues, our results became more consistent. By mid to late 2007, we had increased the number of instruments, and although the instruments themselves were becoming more reliable, we were facing considerable issues of consistency in quality as we scaled up. We began a very focused quality-control program in late 2007. This, in combination with improvements to the instruments and chemistry, has led to a dramatic improvement in sequencing capabilities (Fig. 1).

In addition to building our sequencing capacity, we had to develop methods to target areas of the genome as well as to use the sequencing capacity efficiently. One of the problems with using the instruments efficiently was that the sequencer produces much more data than are needed in many cases. Thus, the cost of sequencing a sample is much greater than it need be. To solve this problem, we developed a method to bar-code samples. We developed custom adaptors for sequencing that have a molecular bar code imbedded in them in addition to the sequences required for Illumina sequencing. This allows us to make libraries from multiple patients with a distinct bar code associated with each patient. We can then pool the patient samples in a single lane for sequencing and, when the data are obtained, use the bar-code sequences to disambiguate the data and associate them with the proper input patient sample. This has allowed both a significant increase in speed and a decrease in cost per patient on the DISC1 (disrupted in schizophrenia 1) project. As an example, in addition to allowing the technicians in the lab to process more samples, bar coding has cut the cost of

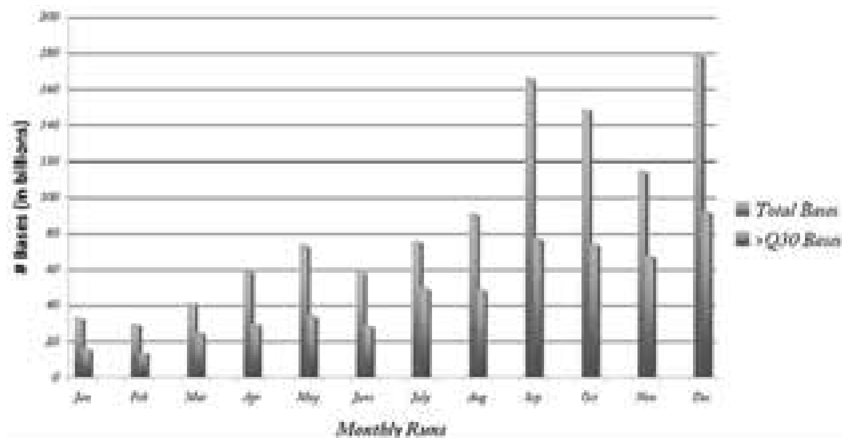

FIGURE 1 Comparison of total bases generated versus total high-quality bases for monthly Illumina runs from August 2007 through mid January 2009. Note that output drops during partial weeks around the Thanksgiving and Christmas–New Year's holidays. We use the quality measure of Q30 to indicate a very high-quality sequence for the purposes of pushing the limits on quality. Much lower-quality bases are still quite useful, but focusing on the more stringent Q30 metric provides constant pressure to improve production quality.

sequencing reagents for a single DISC1 patient from about $800 to about $165. Without working to achieve such savings, we would not be able to sequence adequate numbers of individuals.

Analysis of Variation in the Region of the DISC1 Gene

J. Parla, E. Ghiban, S. Muller, M. Kramer [in collaboration with D. Porteous, D. Blackwood, W. Muir, and I. Deary, University of Edinburgh, and P. Visscher, University of Queensland]

One method for isolating specific genomic regions for sequencing is to use polymerase chain reaction (PCR). Improvements to PCR through the discovery and engineering of DNA polymerases with distinct activities that can meet different experimental requirements, as well as through the development of PCR additives that enhance the capabilities of PCR, have enabled the efficient amplification of challenging targets. In particular, the availability of DNA polymerases that can readily amplify targets >10 kb has been critical for improving the feasibility of projects that require the amplification of large regions of the genome from large groups of individuals.

During our investigation of the feasibility of long-range PCR for targeted resequencing, we evaluated several DNA polymerases marketed for the amplification of long targets. Our minimal requirements included reliability, robustness, and scalability. As a result of our screening efforts, we are now routinely using rTth DNA Polymerase, XL (Applied Biosystems) in a production-level setting. rTth DNA Polymerase, XL is actually a blend of a polymerase with high processivity and a polymerase that confers fidelity due to its 3′-5′ exonuclease activity. The proofreading activity is important for minimizing nucleotide misincorporations that might lead to premature termination of DNA synthesis.

The nature of our target amplification using long-range PCR is also a result of primer design. We use Primer3 software to design our primers based on the specifications that we require for optimal target amplification. Our targets are covered in a tiling fashion using primer sets that have an ~1-kb overlap. Following the separate PCR amplification of each section of a genomic target, the reactions performed for each individual are pooled using normalization to promote even coverage of the target. Through our optimization of long-range PCR, we have also determined that it is critical to remove any primer-dimer artifacts produced during amplification. Primer-dimers that are particularly large are fully viable sequencing substrates, and we found that significant levels of primer-dimers dramatically reduce the actual coverage of target regions corresponding to the primer sequences. We use AMPure (Agencourt) to remove primer-dimers from amplicon pools before sequencing, thus allowing us to obtain more even and more accurate target coverage and to reduce the sequencing wasted on nontarget material.

In our DISC1 project, we have been working on the resequencing of an ~550-kb region of human chromo-

some 1 that includes DISC1. This region has been strongly implicated in cognitive disorders by our collaborators Drs. Porteous, Blackwood, and Muir (St Clair et al., *Lancet 336:* 13 [1990]; Millar et al., *Hum. Mol. Genetics 9:* 1415 [2000]). Our initial study included 40 DNA samples from patients diagnosed with schizophrenia and from normal controls. These sequencers were very new. The goals of this initial study were severalfold: What was the best way to prepare samples for sequencing? What were the best ways to analyze data? What sequence coverage was necessary for high-confidence polymorphism detection? The purpose of our pilot was to answer these questions as well as to find any other problems that needed to be addressed experimentally or computationally. The results from this pilot study were quite promising, because we were able to cover up to 98% of our target region at 30x or greater coverage depth. We found that a minimum of 20x–30x sequence coverage depth would be necessary for the accurate identification of genetic polymorphisms. The accuracy of the sequencing at that level was confirmed by comparison with data from a subset of the same patients analyzed with different methods in the Porteous lab. We were able to optimize the steps in our process, find elements that we needed to avoid, and place ourselves in a position to confidently scale up the project.

Once we had proven that the targeted resequencing of DISC1 using long-range PCR and Illumina sequencing was possible, we decided to increase this project to a production scale. This began in 2008, and we are currently analyzing a set of 984 DNA samples that represent schizophrenia, bipolar disorder, major depression, and normal controls. To enhance our data sets, we are including a set of 1072 DNA samples obtained by the Lothian Birth Cohort study. This large study was designed to track the progression of mental skills from childhood to adulthood. The breadth of cataloged phenotypic information represented by the Lothian Birth Cohort study will add to our ability to interpret the genotypic data that we are generating for our study of the function of DISC1 in cognition.

We have made significant progress in our large-scale DISC1 resequencing project. We are currently maintaining a throughput of 20 individuals PCR-amplified per day and an average of ~84 individuals sequenced per week using the sample bar coding and multiplexing described above. We have completed the PCR amplification of DISC1 from a total of 768 individuals across both of our sample sets. These samples are now at different stages of sequencing and analysis, ranging from preparation for sequencing to preparation for genetic variation analysis. A summary is shown in Table 1. Our sequencing results have shown that we can effectively multiplex up to five individuals on a given lane for sequencing, which has been able to produce adequate and evenly distributed data. We are frequently able to obtain >90% coverage of DISC1 at 20x depth or better and cover the vast majority of exons. Exons that are not completely covered at our required depth are often only partially missing. These gaps in coverage are likely a result of a low percentage of failed PCRs during the tiled DISC1 amplification, which is sensitive to the quality of the DNA template. We are considering the use of Sanger sequencing to help fill in these coverage gaps. Our sequence coverage of DISC1 from the first ~400 patients that have been analyzed is shown in Figure 2. We estimate that we will complete the sequencing of these samples by spring–early summer 2009.

TABLE 1 Current Status of DISC1 Patient Samples at Each Stage of the Sequencing Pipeline

Patients amplified	768
Libraries completed	695
Patients sequenced	695
Patients base called	613
Patients SNP called	317

We are currently comparing variation analysis programs and will catch up on that final portion of the analysis once a decision has been reached on which method to use going forward.

For polymorphism detection and identification, we have been considering various programs. A few programs are available that can be used for analyzing Illumina data, but we have put the most effort into comparing the use of the program that we developed in-house to Mapping and Assembly with Quality (Maq). We have become increasingly in favor of using Maq for genetic variation analysis due to the dedicated bioinformatics personnel that continually update the program with improvements and the success that other groups have had with the program. At this point, we have just initiated our Maq analysis of the data from our pilot DISC1 study and will compare our Maq results to those we have already obtained using our in-house program.

Developing Tools to Analyze the Genetic Variation in the Synaptome

J. Parla, E. Ghiban, L. Spiegel, M. Kramer [in collaboration with Greg Hannon, HHMI/Cold Spring Harbor Laboratory; E. Hodges and Z. Xuan, Cold Spring Harbor Laboratory; J. Potash and F. Goes, Johns Hopkins University School of Medicine; and S. Grant, Wellcome Trust Sanger Institute]

To effectively use the power of massively parallel sequencing, in terms of both cost and machine time,

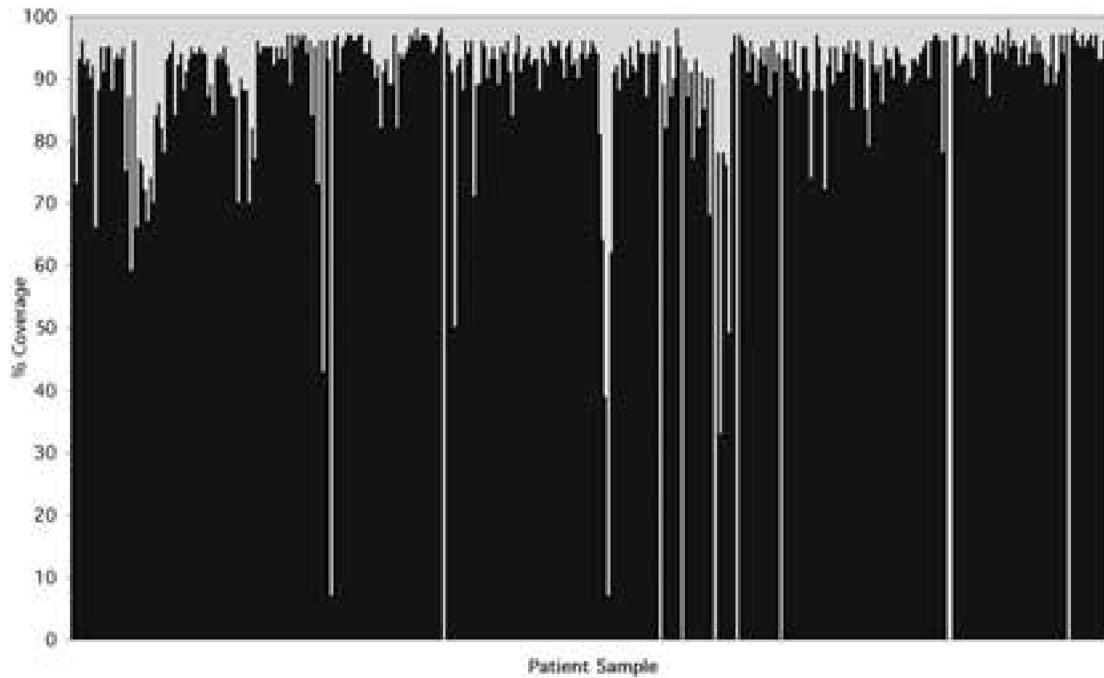

FIGURE 2 Percentage of the DISC1 target region covered in 380 patient samples. The vast majority of the samples have a minimum of 85% of the region covered at a 30x sequencing depth. Samples for which we have obtained significantly <85% coverage of DISC1 at 30x generally represent instances of sporadic issues experienced with sample multiplexing and sequencing, rather than with DISC1 amplification.

complementary tools are needed to maximize their capacity. This can be achieved through methods that direct sequencing efforts to genomic regions of high interest. PCR is suitable for this in some cases, such as DISC1, in which a large contiguous region of introns and exons is being targeted, but it is not appropriately scalable for all applications. Specifically, the Illumina GA was capable of generating nearly 3 Gb of sequence per run. For targets such as small genomes (~100 million bases), this amount of sequence output provides adequate depth and is suitably cost-effective. However, complete shotgun sequencing is currently impractical for the larger and more complex mammalian genomes, and simplification of the DNA fragment population (genome partitioning) is still required to boost coverage depth of the informative regions. We have developed an economical and focused method for genomic target capture and directed resequencing that enables sequence variation to be cataloged throughout large subsets of the nonrepetitive genome. To do this, we exploited the flexibility of highly parallel in situ oligonucleotide synthesis platforms to create a substrate for hybrid selection, and in contrast to reduced representation techniques, our approach is "programmable" for any specified genomic region. Our strategy combines microarray-based hybrid selection with massively parallel sequencing on the Illumina GAII to yield a flexible and practical mutation discovery strategy. These methods are already proving to be highly competitive in both efficiency and cost, in contrast to other target selection techniques including long-range and multiplex PCRs.

We were one of a small number of groups that published a method on targeted genome sequencing at the end of 2007 (Hodges et al., *Nat. Genet. 39:* 1522 [2007]). Subsequently, this has been an area of intense research by multiple groups around the world, and significant effort is being undertaken using the strategy to capture genes in many disease or basic science resequencing projects. We have continued to make very significant improvements to the technique and to maintain a position as one of the leaders in the field. Previous attempts at a "candidate gene approach" for finding genes involved in cognitive disorders have been limited by technology in terms of both the number of genes that can be examined and the number of patients that can be examined. The combination of the new sequencing technology and the hybrid selection approach that we developed and described above eliminates that limitation.

Dr. Seth Grant of the neuroscience group at Cambridge University and the Wellcome Trust Sanger Institute in the United Kingdom has developed a concept that he calls the "synaptome," which entails identification of the proteins that function in the synapse, the basic functional unit of the nervous system. There is considerable reason to believe that at least some of the genes involved with cognitive disorders encode proteins that function in the synapse. First, from a basic biology standpoint, most functions of the neurons are localized at the synapse. Second, some drugs with efficacy in conditions such as depression and bipolar disorder target synaptic components. In fact, many previous targets of psychiatric genetics investigations were synaptic components. In the past, these were genes targeted singly or a few at a time, but now we can target all 1200–1500 genes in the synapse at the same time.

As an initial test, we designed two capture arrays to study ~1200 genes in the synapse (~17,000 exons plus promoters). We performed capture and sequencing (at a coverage lower than necessary) to simply get a basic idea of capture performance. We found that ~95% of the bases in the target were sequenced at least once in the experiment. Following this result, we decided to focus on one of the two arrays while we optimized protocols to increase the speed and efficiency of optimization.

We synthesized one array including one-half of the synaptome genes plus 60 genes that had been associated with bipolar disorder based on genome-wide association studies. The latter set of genes had been specified by our collaborators Drs. James Potash and Fernando Goes of the Mood Disorder Clinic at Johns Hopkins University. We then tested this capture array on the control samples. Selected results are shown in Table 2.

We are still optimizing this array and its use but have started an initial pilot study on 30 bipolar-affected patients from our Johns Hopkins collaborators. We are applying to the National Institutes of Health for additional funding to scale-up this project. Our collaborators are Dr. Potash, his colleagues at Johns Hopkins, and Dr. Grant. Our goal in this project will be to sequence the synaptome of 800 bipolar patients and 400 controls to identify genetic alterations.

Detailed Analysis of Gene Expression in Regions of the Human Brain

M. Chen, J. Parla, Y. Cai, M. Kramer [in collaboration with G. Hannon, HHMRI/Cold Spring Harbor Laboratory; Z. Xuan and M. Zhang, Cold Spring Harbor Laboratory; and R. Yolke, Johns Hopkins University School of Medicine]

The most recent approach that we are implementing is the sequencing of cDNAs and small RNAs from the brains of normal and affected individuals. There are several reasons for pursuing this approach, although it is limited by the availability of samples. We will be able to look for mutations in the coding regions of large numbers of expressed genes in each patient (5000–10,000). We will also be able to look for changes in gene expression, possible fusion genes, and gene splicing changes with these data.

The role of small RNAs has only recently been appreciated. They are quite important in some types of development and thus of interest due to the likely developmental origins of cognitive disorders. Our work would be one, if not the first, large-scale examination of small RNAs from the brains of patients with cognitive disorders.

Finally is the value of deep-brain cDNA sequence data in the search for infectious agents that might be the environmental trigger of cognitive disorders. Deep

TABLE 2 Statistics for Target Capture of Coriell Control Sample NA19775 with Increasing Numbers of Reads

	Capture of Coriell sample NA19775								
	1 lane	2 lanes	3 lanes	4 lanes	5 lanes	6 lanes	7 lanes	8 lanes	9 lanes
Total bases of targets on chips	3,327,512 bp								
Number of target regions	9338 (exons plus promoters)								
Percentage (%) of targets covered at 1x	92.0	93.6	93.9	94.1	94.2	94.3	94.3	94.4	94.4
Percentage (%) of targets covered at 20x	18.3	53.6	68.0	74.5	78.9	81.2	82.8	86.3	87.1
Theoretical enrichment	826								
Experimental enrichment	337	336	335	334	334	338	338	337	336
Efficiency (%)	40.8	40.7	40.6	40.4	40.4	40.9	40.9	40.8	40.7

Target regions include exons and promoters. Target regions were combined if the distance between two regions was <120 bases. Capture efficiency is ~40%. There is nearly complete coverage of the target at 1x following a single lane of sequencing. In contrast, target coverage at 20x increases in a nonlinear fashion and reaches 87% merging nine lanes. Note that this uses one sample as an example and there were three successfully multiplexed samples in this set of sequenced samples, so in these lanes, other samples also were sequenced to significant coverage. We are still optimizing bar codes and protocols to make the sequence coverage more evenly distributed among multiple samples and to eliminate failed bar codes that occasionally occur.

sequencing of the cDNAs from the brain is probably the best way to detect the presence of such pathogens. Any pathogen in the brain will likely make RNA transcripts, and if transcripts of the same or related pathogens are known, we should be able to detect them by searching the public databases for the sequences that we detect. We have shared all of our data with Bob Yolken and colleagues at Johns Hopkins and will continue to do so in order to assist them in their search for environmental factors that might be triggering the disorders.

mRNA from three brain samples were obtained from the Stanley Medical Research Institute for a deep sequencing of cDNA and small RNA pilot project. For each of the samples studied, poly(A) mRNAs were isolated, and the purified mRNA samples were fragmented chemically. Double-stranded cDNAs were synthesized using reverse transcriptase for the first strand and DNA polymerase I for the second. Following purification, the samples were converted into cDNA libraries using a series of steps derived from the Paired-End Sample Prep Kit (Illumina), with several modifications. Two rounds of 76-cycle sequencing were conducted on each sample.

Reads were aligned back to full-length transcripts first. Reads that had no matches in this step were collected and aligned back to the human genome and in-house splicing junction probes. The junction probe set was generated by concatenating 76 bp immediately upstream of the 5′ end of an exon and 76 bp immediately downstream from the 3′ end of another exon. Alignment quality scores were used to choose the best alignment among reads that were mapped to multiple locations.

TABLE 3 Summary of Sequencing Results on Separate Transcriptome Samples

	Sample D-5	Sample D-16	Sample D-42
Sequencing methods		Paired-end 76 cycles	
Number of single reads used to identify cDNAs	7,905,719	14,928,681	13,851,716
Number of genes hit	17,778	17,920	18,189
Number of genes with 90% exon coverage at 5x depth or higher	1855	1632	2540

Paired-end sequencing for 76 cycles were conducted. After two separate lanes in each patient were merged, 8–14 million reads were used to identify cDNAs. The detected genes herein include known and novel genes.

Among the three samples we studied, an average of 12,228,705 reads from two separate lanes were used to identify cDNAs. Our analysis suggested that of the 17,962 genes to which the reads aligned, an average of 1925 known genes and 94 novel genes can be covered across 90% of their length and at fivefold or higher depth (Table 3). Studies on the distribution of splicing events indicates that 40% of reads carry information from one exon, ~13% bridge two exons, 11% from intronic elements, and 24% from intergenic elements (Fig. 3). Known and functional noncoding RNAs are detected by ~2% of the reads. Distribution of splicing events in a normal brain sample will be conducted in the near future.

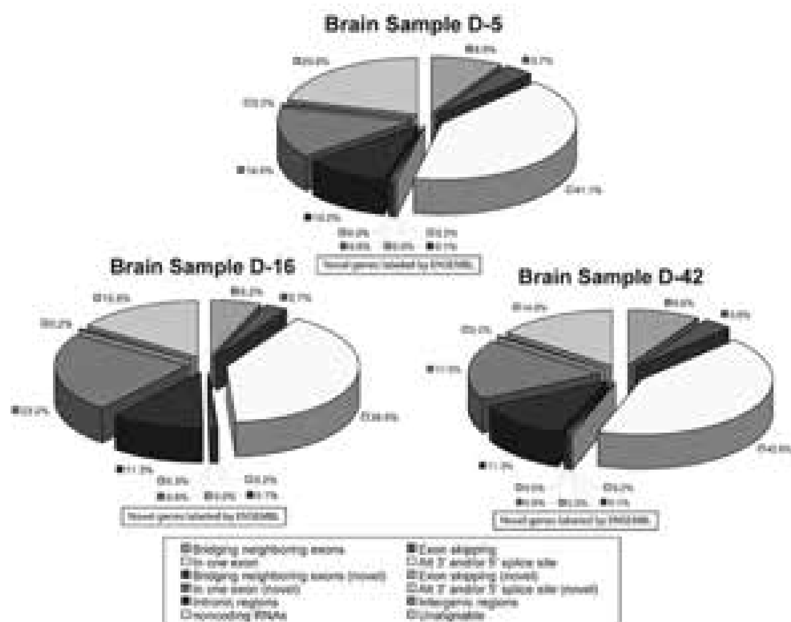

FIGURE 3 Distribution of splicing events among reads remains similar among samples. This distribution was extrapolated using two alignment tools: ELAND and BLAT. The former uses stringent conditions and is aware of quality, and the latter is less restrictive. There are ~2000 genes that are detected, and ~5% of these are novel genes.

CANCER GENES

S. Powers A. Bakleh K. Liu E. Sawey N. Sullivan
C. Cai J. Marchica A. Song J. Zhang
M. Chanrion M. Rajaram M. Su

We are interested in the identification and functional characterization of cancer genes (oncogenes and tumor suppressors). Our main motivation for studying cancer genes is their proven practical value in serving as targets for new cancer therapies and as biomarkers that can guide treatment decisions. Additionally, cancer genes have normal functions and their characterization can often lead to a deeper understanding of basic biology.

ONCOGENOMIC cDNA SCREEN

We have continued our systematic functional analysis of candidate amplified oncogenes in liver cancer. To do this, we have leveraged the National Institutes of Health (NIH)-funded genome resource of sequence-verified human cDNA expression vectors (the Mammalian Gene Collection). From a select group of genes that are highly amplified in human liver cancer samples, we have transferred cDNAs into retroviral expression vectors. We started with 29 recurrent amplicons, including the well-known *CCND1*, *MET,* and *CDK4* amplicons, that we had identified from the data set of 106 human hepatocellular carcinoma (HCC) samples analyzed by ROMA (representational oligonucleotide microarray analysis). From the total set of genes within these amplicons, we obtained 126 sequenced-verified cDNA expression vectors. We used retroviral transfection to introduce cDNA expression vector pools into mouse liver epithelial cells (PHM1) and assayed for tumor formation. The cDNAs responsible for positive hits were identified and subsequently validated by individual transfections. Of the 126 cDNAs, 18 scored positive by this criteria, including *CCND1*, *MET*, and *CDK4*. In contrast, none of 24 control cDNAs—chosen at random from the NCBI RefSeq set—scored positive for tumorigenicity. We are currently assaying a larger set of control cDNAs to obtain a better estimate of the background rate of the tumor-promoting function in this system. Besides identification of *CCND1* from the 11q13 amplicon, we identified two oncogenes from a recurrent amplicon located at chromosomal region 20q.11: *HCK*, which has been shown to be oncogenic in hematopoietic cells, and *POFUT1*, a gene involved in Notch signaling that has not been shown to be oncogenic in any system. We also identified as oncogenes two largely uncharacterized genes, one encoding a zinc finger protein, *ZCCHC7,* and the other a potential element of basal transcriptional machinery, *POLR1C*.

CHARACTERIZATION OF THE NOVEL ONCOGENE *FNDC3B*

We decided to drill down further with one of the novel oncogenes discovered in the oncogenomic screen, *FNDC3B*. This gene, located on the q arm of chromosome 3, is frequently amplified and overexpressed not only in human liver cancer, but also in lung, pancreatic, and breast cancers. Its expression is associated with metastatic spread and poor prognosis in breast cancer. There are only two papers in the literature describing *FNDC3B:* one on its identification as a transcript that is turned on during the early stages of adipocyte differentiation and the other describing its requirement for postnatal survival of mice and a preliminary investigation of cellular phenotypes deficient in *fndc3b*$^{-/-}$ mouse embryo fibroblasts. We have focused on determining how *FNDC3B* overexpression can cause malignant transformation. Thus far, we have determined in three different epithelial cell types that *FNDC3B* overexpression malignantly transforms cells, induces the epithelial–mesenchymal transition by both molecular and morphological criteria, and activates Akt. What is unusual about the latter finding is that overexpression only affects phosphorylation of serine residue 473 and not threonine residue 308. This is unlike growth factor activation of Akt; instead, it resembles the effects of overexpression of *Rictor,* the activating component of mTORC2. The correct localization of FNDC3B may be necessary for this Akt-activating function because several mutants that cause mislocalization also block Akt activation (Fig. 1).

We found that silencing expression of *FNDC3B* by RNA interference (RNAi) suppresses tumorigenicity of human cancer cell lines that have increased gene copy number and expression. Thus, it clearly has an active role in tumor maintenance, and further study of its oncogenic function could provide valuable new insights into both its normal biological role and strategies for therapeutically counteracting its oncogenic effects.

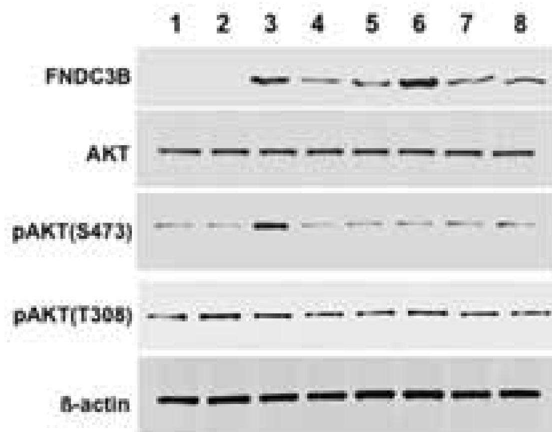

FIGURE 1 Effect of overexpression of FNDC3B and FNDC3B mutants on the activation status of Akt. Labels at left indicate the antigen detected by the indicated immunoblot. (Lanes 1 and 2) Untransfected and empty vector control hepatocytes; (lane 3) wild-type FNDC3B; (lanes 4–8) five different mislocalization mutants of FBDC3B.

CHARACTERIZATION OF EPIGENETIC ALTERATIONS IN LIVER CANCER

In the past, we have studied genetic alterations, specifically DNA copy-number alterations, as our main approach to exploring cancer progression. However, it has become increasingly clear that many of the important alterations that occur during cancer development are epigenetic. In the past few years, powerful new methods for epigenomic profiling have been developed using both array and single-molecule sequencing platforms. Using these new platforms, we have begun epigenomic profiling of both human and mouse liver cancers and will look for regions that are commonly altered during cancer progression. We have also reanalyzed our extensive data set of expression alterations that occur in both human and mouse liver cancers to look for statistically significant clusters of genes that show regional alterations suggestive of underlying epigenetic alterations. This approach is already yielding candidate loci that are commonly altered in both human and mouse liver cancers that are currently being tested for epigenetic changes as well as the presence of functional cancer genes.

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GENE-COPY-NUMBER VARIATION AND ITS ROLE IN HUMAN DISEASE

J. Sebat M. Kusenda S. McCarthy
V. Makarov V. Vacic
D. Malhotra S. Yoon

The major focus of our laboratory is to identify genetic causes of mental illness by analysis of rare copy-number variants (CNVs) in the human genome. We have developed an experimental design that allows us to identify rare mutations that confer high risk for disease. Our approach is to screen the genomes of patients with schizophrenia and bipolar disorder to identify deletions and duplications of DNA that disrupt genes. These mutations are subsequently tested for association with disease in families and in large samples of patients and healthy individuals. This approach is based in part on the findings of our previous genetic studies of autism spectrum disorders (ASDs), which established an important role for rare, spontaneously occurring CNVs in the etiology of ASDs. We have now successfully applied this approach to studies of schizophrenia and bipolar disorder. Our findings, coupled with studies by other groups, have shown that rare CNVs have an important role in the genetics of psychiatric disorders. Finally, most individual CNVs that have been definitively identified to date increase risk of mental illness by ≥10-fold, strongly suggesting that these mutations have a causal role.

We have begun to enter a period of rapid discovery. The key challenges that lie ahead include (1) capturing a greater fraction of the rare genetic causes of bipolar disorder and schizophrenia, (2) understanding the clinical and biological effects of each individual mutation, and (3) determining the functions of the individual genes and how these different genes interact within the context of cellular pathways. Such a unified understanding of the biology of bipolar disorder and schizophrenia will require integrated efforts in clinical psychiatry, neuroscience, genetics, and computational biology.

Analysis of Copy-number Variation in Schizophrenia

J. Sebat, S. McCarthy, D. Malhotra, M. Kusenda, S. Yoon

Our studies of CNVs in psychiatric disease have revealed new insights into the genetics of mental illness. Earlier this year, we published a paper demonstrating that rare deletions and duplications that impact genes are significantly more frequent among individuals with schizophrenia than among controls; these CNVs affect genes involved with neural development. In addition, genomic regions have now been identified where individual deletions or duplications of genes are strongly associated with psychiatric disorders. This research, coupled with similar findings in neurodevelopmental disorders such as autism and mental retardation, suggests that genetic architecture of mental illness includes a large constellation of rare severe mutations in many different genes.

MICRODUPLICATION OF 16P11.2 IS ASSOCIATED WITH SCHIZOPHRENIA

Since the publication of our initial findings, we have made rapid progress toward identifying individual mutations that confer high risk for schizophrenia. We have definitively identified a microduplication of 16p11.2 as a strong risk factor for schizophrenia in a sample of 1922 cases and 4062 controls ($P = 5.3 \times 10^{-5}$, OR [odds ratio] = 12.8) and in a replication sample of 2645 cases and 2420 controls ($P = 0.022$, OR = 8.3) (Table 1). Interestingly, we confirmed earlier reports of a strong association of the same mutations with ASDs and a weak association with bipolar disorder. Taken together, these findings suggest that different psychiatric disorders may share common genes (or even the same mutation).

Our findings further strengthen evidence demonstrating a role for rare copy-number variants in schizophrenia. Collectively, these studies provide strong evidence that the illness is characterized by marked allelic and locus heterogeneity. The fact that a mutation is present in only 0.3% of cases does not negate its potential relevance to the broader patient population. Schizophrenia is characterized by marked genetic heterogeneity. Any single mutation or locus will account for only a small fraction of the overall genetic risk. However, in toto, rare mutations may contribute in a substantial portion of overall risk. Furthermore, multiple rare mutations will likely impact the same or related neurobiological systems or pathways. Characterizing these critical brain processes will contribute substan-

TABLE 1 Significant association of 16p11.2 duplications with schizophrenia

	Copy-number state			Frequency	OR	Fisher
	3	2	1			
Discovery data set						
schizophrenia	12	1908	1	0.63%	12.8	5.3×10^{-5}
controls	2	4060	0	0.05%		
Replication data set						
schizophrenia	9	2636	0	0.34%	8.3	0.022
controls	1	2418	1	0.04%		

Duplications of the 16p11.2 critical region (chr16:29558000-30107000, hg18 coordinates) detected by outlier median Z-score clustering in the Discovery data set show significant association with schizophrenia. Odds ratios (OR) and Chi-square tests are based on copy number 3 verses the sum of copy numbers 2 and 1. Duplications are also observed significantly more in Replication data set cases than in controls.

tially to our understanding of pathophysiology and provide important targets for treatment development.

A Family-based Study of Rare Structural Variants in Bipolar Disorder

J. Sebat, D. Malhotra, M. Kusenda, S. Yoon, S. McCarthy

Our research in bipolar disorder aims to make a significant contribution to the field in two ways: by improving the scientific understanding of bipolar disorder through genetic studies and by creating a novel genomic resource for geneticists. To these ends, we have initiated a genetic study—the Genetics of Early Onset Mania (GEM). The goals of our genetic study of bipolar disorder are to (1) perform genome-wide analysis of copy-number variation in bipolar families, (2) assess the overall contribution of de novo and inherited mutations in sporadic and familial bipolar disorder, and (3) identify novel candidate genes for further study.

Although it has been established that rare de novo mutations contribute to the etiology of autism and schizophrenia, it is not currently known whether de novo CNVs have a significant role in bipolar disorder. On the basis of the existing studies, it is clear that rare de novo CNVs occur frequently in children with severe impairments in cognitive development. We do not know if the same will necessarily be true in patients with mood disorders (but otherwise normal intelligence). Therefore, we have initiated a study to test this hypothesis empirically.

To properly investigate the role of de novo mutation in bipolar disorder, researchers must have access to DNA samples from “trios,” where a trio consists of a single patient with bipolar disorder and a mother and father with no immediate history of mental illness. Currently, there are many family samples available from public and private collections; however, because these samples were initially assembled for the purpose of linkage analysis, very few sporadic cases were collected. Consequently, these collections provide few trio families needed for our study. Thus, through the work of three clinical groups at Zucker Hillside Hospital, Johns Hopkins University, and the National Institute of Mental Health, we have begun a collection of new bipolar families. The GEM sample is unique from most existing DNA collections in two ways: First, a significant fraction of patients are “sporadic” cases, and second, approximately half of patients in the collection have an early age-at-onset (age at first mania <18). This collection will be made available to the scientific community, where it will become a valuable resource for scientists interested in studying genetic and epigenetic causes of sporadic or young-onset bipolar disorder.

Sample Collection. The collection of new bipolar families has been steady, and recruitment has increased at some sites due to greater publicity surrounding this project. The current sample consists of 785 individuals from 263 families.

Preliminary Results. For these studies, we are using a higher-resolution CNV detection platform that detects CNVs that are as small as 10 kb. Thus, we are able to reliably detect 120 CNVs per genome on average, more than twofold more than can be detected with any other platform including the Affymetrix 6.0 and the Illumina 1M. To date, whole-genome scans have been performed on 518 samples from 191 families (146 of which are complete mother-father-child trios). The remaining 72 families will be completed in spring 2009. We have begun preliminary analyses of these data in three ways: (1) de

novo mutations in trios, (2) inherited CNVs in trios, and (3) rare CNVs in a large sample of cases and controls. Our preliminary results find evidence for a higher rate of spontaneous mutation in sporadic bipolar disorder as compared to healthy controls. Although we need to apply our analysis to the larger sample in order to gain statistical support for our findings, the preliminary results suggest that de novo CNVs contribute to bipolar disorder.

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A. Bhandari

GENOME-SCALE DATABASES OF PATHWAYS, GENETIC VARIATION AND EVOLUTION

L. Stein S. Avraham, S. Cain, N. de la Cruz, B. Faga, X. Feng, G. Gopinathrao, T. Harris, A. Kanapin, S. McKay, W. Spooner, P. van Buren, L. Wang, G. Wu, Z. Zha, W. Zhao

THE HUMAN HAPLOTYPE MAP

The International Human HapMap Project (www.hapmap.org) is an international project to map out regions of common genetic variability in the human genome by genotyping three major world populations across a large number of naturally variable sites. 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 website, 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 the past 2 years, the HapMap website has been used for several hundred whole-genome association studies that have identified novel genes implicated in type-1 and -2 diabetes, hypertension, rheumatoid arthritis, tuberculosis resistance, cardiovascular disease, inflammatory bowel disease, and other medically significant traits.

During 2008, we enhanced the HapMap website to provide information on genetic variation in an additional six human populations, including populations originating from the Indian subcontinent and Mexico. This will help researchers to identify genetic risks that are specific to specific subpopulations. We have also built new visualization and analysis tools into the website itself.

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 Wellcome Trust Sanger Institute in the UK. Our lab is responsible for the website, user interface, and software architecture for the project.

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

During 2008, we added information on several newly sequenced nematodes, providing researchers with the ability to identify regulatory sites that have been conserved over evolutionary time.

REACTOME

Reactome (www.reactome.org) is a collaboration with the European Bioinformatics Institute (EBI) and the Gene Ontology Consortium to develop a web-accessible resource for curated information about biological processes.

Reactome is organized like a review journal. Bench biologists are invited to create modules that summarize a particular aspect of their field. Currently, summations include DNA replication, transcription, translation, intermediary metabolism, the cell cycle, RNA splicing, and hemostasis. Many more modules are under way. Modules are similar to mini-reviews, except that each paragraph of text is reduced to a series of logical assertions that is entered into a database of processes and macromolecules. The database is then used to drive a website that can be browsed like a textbook or searched with queries to discover pathways and connections.

During 2008, we brought the number of genes curated in Reactome to 3368 proteins and variants, covering ~15% of the annotated portion of the human

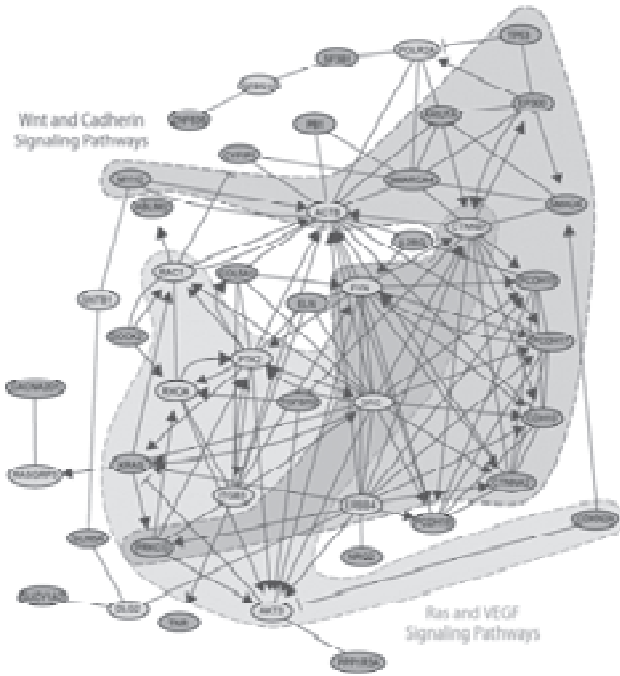

FIGURE 1 Reactome provides a tool for interpreting cancer genome resequencing studies. In this figure, we have mapped out the interactions among genes that are repeatedly mutated in pancreatic cancer. Many of these genes are members of classic cell growth and regulation signaling pathways, and many are also mutated in another type of cancer, glioblastoma multiforme. Genes that are particularly heavily interconnected, such as *ACTB*, may be key components of a shared cancer pathway.

genome. We supplemented this data set with functional interaction data from microarrays, protein interaction experiments, and other sources to create an integrated network of functional interactions covering ~50% of the genome. This integrated network is the largest one published to date, containing ~200,000 known and predicted gene interactions. We have used this network to interpret the results of several large-scale genome-resequencing efforts for the brain tumor glioblastoma multiforme and pancreatic cancer and have identified a core set of protein interactions common to both cancers (Fig. 1). This suggests the presence of a shared cancer pathway and points to several genes that may be involved in the development and progression of these cancers. A paper describing these results is now under review.

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PLANT GENOME ORGANIZATION, EVOLUTION, AND FUNCTION

D. Ware S. Avraham, J. Chia, A. Eveland, B. Hurwitz, X. Liu, L. McMahan, A. Narenchania, S. Pasternak, L. Ren, W. Spooner, J. Stein, J. Thomason, P. van Buren, K. Youens-Clark, L. Zhang

COMPARATIVE GENOMICS: A MODEL FOR UNDERSTANDING FUNCTION THROUGH EVOLUTION AND GENOME ORGANIZATION

The cereal genomes are believed to have diverged from one another within the past 50–80 million years (Ma). During this time, there have been numerous genomic rearrangements that have occurred in cereal genomes, such as segmental and tandem duplications, polyploidization, and transposition. This is especially true for maize, which has undergone tetraploidization and is known to have many recent modifications to its genome structure due to transposable elements. Previous studies have shown that regions of conserved protein-coding gene order between cereals represent functionally important regions and potentially conserved phenotypes.

The transfer of information across and within species through comparative mapping, integration of genomic sequence, genetic and physical maps, and phenotypes adds significant value to existing genome-sequencing and -mapping studies. As part of this work, our group contributes to the infrastructure of Gramene (http://www.gramene.org), a collaborative that leverages sequence and functional information from models and sequenced plant genomes for translational genomics in agriculture. In the last year, our group has completed several milestones on the Gramene project including the release of the 28th and 29th builds since 2000. Of the many improvements made, we added to our Ensembl Genome Browser nine sequence genomes of agronomically and scientifically important species. Our BioMart data warehouse, used for bulk data downloads and data mining, has been expanded to encompass mappings, markers, ontologies, and quantitative trait loci (QTL). This improvement allows users to combine searches on both phenotypes and sequences. We have improved our gene trees and enhanced searches for our markers and sequence database by ontology terms and tags. Auto-complete input boxes will make useful suggestions on user searches, and we have added a new Gramene News blog and RSS (Really Simple Syndication) feed for users to stay abreast of our latest news. Several new web services allow more direct access to our databases including distributed annotation servers (DAS) for Gramene and Ensembl genes, QTLs via Simple Semantic Web Architecture and Protocol (SSWAP), and a public, read-only MySQL server hosting Ensembl, QTL, and genes and markers/sequences databases.

SEQUENCE AND ANALYSIS OF THE MAIZE B73 GENOME

From its domestication 8000 years ago in Central America to its position today as the world's leading harvested grain, maize has had an important role in human civilization, providing food, animal feed, and biofuel. Maize also enjoys a long and distinguished history as a model organism owing to its rich diversity and tractable genetics. The complete sequence of the maize genome would therefore propel advances in basic research as well as in agriculture and other industries.

The Maize Genome Sequencing Consortium was launched with a 3-year grant from the National Science Foundation (NSF) to produce a complete sequence of the maize "B73" genome. At 2.5 Gb, the maize genome rivals that of the mammalian genome in terms of size and is six times larger than that of rice because of its high content of retrotransposable elements. To meet the challenge of producing a draft sequence, the consortium took a hierarchical approach, selecting a minimal tiling path of bacterial artificial clones (BACs) from a 20x fingerprint map and sequencing the BACs using a combination of shotgun and finishing of the unique regions of the BACs. Our lab participates with Drs. McCombie, Stein, and Martienssen in the Maize Sequence Consortium at CSHL. Now in its third year, the project has produced complete sequences of 16,700 BAC clones comprising ~2.8 billion nonredundant bases, all available via GenBank.

In the past year, our laboratory has produced repeat and gene annotations for BAC sequences that account for 95% of the maize genome. Preliminary analysis suggests that 79% of the maize genome is repetitive. The data sets can be visualized as chromosome-level distributions. The view is very similar to a Google map view zoomed out, where we can see trends in the genomic

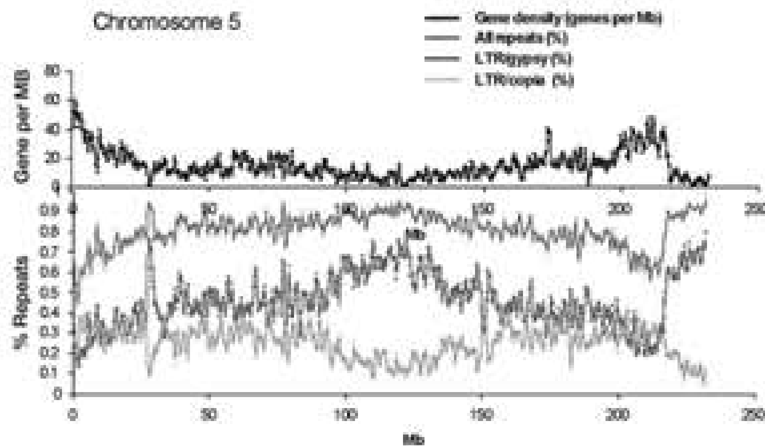

FIGURE 1 Maize chromosome 5 distribution of genes and repeats.

landscape. At the chromosome level, we observe that genes and repeats are not evenly distributed, and there is a direct inverse correlation between the densities of two types of sequences: When gene density increases, repeats decrease. Overall, we find that the ends of the chromosome tend to be more gene rich but in a few examples, such as chromosome 5, we find that the end regions are gene-poor (Fig. 1).

Until the end of this year, we have not had access to the complete genome sequence, but we did have access to the completed region on maize chromosome 4, where we were able to begin some detailed analysis. From this region, we were able to generate detailed comparative maps between rice and maize and rice and sorghum, which reveals that the maize genome has been largely shaped by its history of tetraploidization subsequent to rearrangement and duplicate gene loss (Fig. 2). Using genes that were conserved among rice, sorghum, and maize in this region, we were able to determine that the overall exon size has remained the same in the three genomes; however, the intron size in maize has increased, due to insertion of repetitive DNA. The sequence and gene annotations are available at the project website's genome browser (http://www.maizesequence.org/).

ASSAYING MOLECULAR AND FUNCTIONAL DIVERSITY IN PLANT GENOMES

Dissection of complex traits in higher eukaryotes still remains one of the major challenges in biology. Currently, dissecting complex traits involves either the identification of large genomic regions or the testing of one gene at a time. At the heart of both approaches is a requirement for segregating markers that are easily scored and constructing appropriate populations.

Next-generation sequencing technologies enable the sequencing of genomes at deep coverage, providing a cost-effective means to identify genome-wide variation. In the last year, we have developd an automated pipline for single nucleotide polymorphism (SNP) discovery from such sequencing data and implemented it to identify genetic variation in two important agricultural crops: maize and grape.

In grape, we identified 470,000 SNPs, and from these, we selected a set of 10,000 SNPs that best repre-

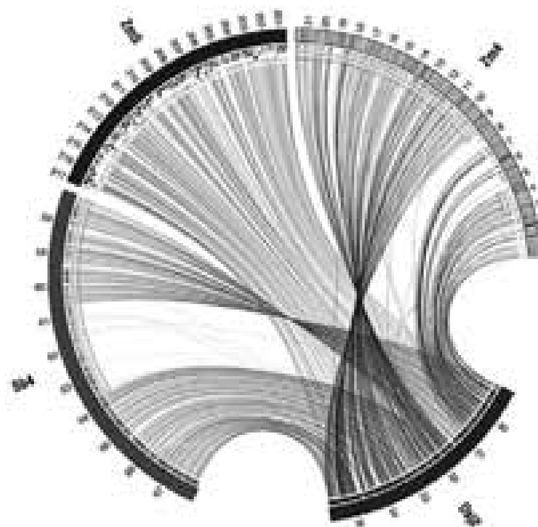

FIGURE 2 Comparative mapping of protein-coding genes in orthologous segments of rice, sorghum, and maize. (Osj2) Rice (japonica) chromosome 2; (Sb4) sorghum chromosome 4; (Zm4) maize chromosome 4 (ctg182 assembly); (Zm5) maize chromosome 5 (homeologous region). Mappings of protein-coding genes are based on the reciprocal best hit.

sent the genetic diversity across domesticated grape varieties as well as related wild species. These 10,000 SNPs will be used as genetic markers to survey the USDA's germplasm repository, providing valuable information for mapping traits such as disease resistance and ultimately decreasing the crop's heavy reliance on fungicides. We have also analyzed almost 1 billion reads from 27 diverse maize lines where we identified more than 5 million SNPs. These SNPs will allow us to characterize the genetic differences across domesticated maize varieties as well as wild relatives to identify traits of agronomic importance.

REGULATORY NETWORKS IN PLANTS

Plant genomes contain thousands of protein-coding and noncoding genes that are expressed throughout the life cycle of the plant. microRNAs (miRNAs) and transcription factors (TFs) are primary regulators of differential gene expression in development and response to stress. TFs bind to DNA *cis* elements to physically repress or activate transcription, whereas miRNA repression acts posttranscriptionally through interaction with complementary mRNA. There are several examples in plants that involve relationships between these regulators and the importance of their role in vegetative growth. *Teosinte branched1* (*tb1*) is a recessive mutant of maize that affects plant architecture. The *tb1* gene was cloned and identified as a transcription factor and a putative miRNA target. More recent work has shown that miR156 and miR172 are involved in a plant phase change from juvenile to adult phase. The dominant *corngrass1* mutant in maize was identified as an overexpression of miR156, which maintains vegetative traits, whereas miR172 levels are low. In our group, we are focusing on identifying regulatory network components and their links. In the last year, we computationally predicted miRNAs from the genomes of sorghum and maize based on conservation of sequence and secondary folding structure. To establish confidence in these predictions, we look for expression-based support of the full miRNA transcript as well as the mature 22-nucleotide miRNA. We can better understand ancestral relationships among these noncoding genes by looking for signatures of conserved genomic locations using orthologous protein-coding genes as anchor points for synteny. Preliminary analyses between maize and sorghum suggest that more than 55% of these maintain a syntenic conserved order in these genomes. Through the study of plant regulatory networks, we will provide researchers and breeders resources to modify plant developmental profiles and responses to stress that are needed to meet the challenge of global environmental changes and land allocation for agriculture in the upcoming years.

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QUANTITATIVE BIOLOGY

Mathematical and statistical insights are at the very heart of the technologies that have made possible comparative genomic studies pioneered at CSHL. Thanks to the foresight of two major private donors, James Simons and Henry "Hank" Greenberg, 2008 marked the unofficial launch of a new Center for Quantitative Biology (CQB), which in the coming year will take up residence in a new building in the Hillside Research Complex. This new research focus at CSHL, under the interim leadership of Michael Wigler, reflects the importance of math and statistics in the forging of new approaches to problems and new ways of understanding the results of biological experiments. The program is bringing together under one roof some of the world's most gifted mathematical minds, who will apply their insights in the formulation of research hypotheses pertinent to the study of molecular biology. Beyond individual research applications, the work of CSHL's quantitative biologists has the potential to revise fundamental notions of how we think about data itself.

Gurinder S. "Mickey" Atwal and colleagues are applying insights from the physical sciences to the study of biological phenomena. Specifically, they develop and use mathematical and computational tools to address quantitative principles governing the behavior of correlated "many-body" biological systems. Such systems range from molecular interactions in a single cell to the evolution of *Homo sapiens*. They are now seeking to understand evolutionary forces acting on the genome in the context of human diseases. In collaborations with colleagues at the Institute for Advanced Study at Princeton University, Atwal has modeled the process by which genetic variants, or alleles, evolve. This has recently led to surprising insights about the role that *p53*, a master tumor-suppressor gene, has in reproduction. This work also bears on the question of demonstrating recent selective pressures acting on our genomes.

Partha Mitra seeks to develop an integrative picture of brain function, incorporating theory, informatics, and experimental work. His theoretical interests are primarily in formalizing the treatment of biological function using ideas and methods from engineering. In informatics work, his lab is developing computational tools for integrative analysis of neurobiological data, spanning electrophysiological, neuroanatomical, and, more recently, genomic data, from multiple species pertaining to the brain. Mitra has organized the Brain Architecture Project, a multi-institutional effort to curate information from the literature about human neuroanatomical connectivity that will also advocate for large-scale studies of connectivity in model organisms. This past year, the lab launched http://brainarchitecture.org, a website featuring full text search for more than 55,000 papers. Separately, Mitra and colleagues progressed in their efforts to develop experimental methodologies for postmortem human brain-tract tracing, a means of tracing neuronal connections in brain areas more rapidly and over greater distances than previously possible. Mitra also recently presented results of neurobiology research demonstrating the de novo evolution of song culture in zebra finches, starting from an isolate population.

Michael Wigler's group uses methods for comparative genome analysis to study cancer and human genetic disease. These methods (called representational oligonucleotide microarray analysis [ROMA] and methylation-specific oligonucleotide microarray analysis [MOMA]) evolved from an earlier technique called RDA (representational difference analysis), used to find tumor suppressors, oncogenes, and pathogenic viruses. Current microarray-based techniques, including comparative genome hybridization (CGH), reveal changes in the numbers of copies of sections of the genome and regions of deletion and duplication, mutations that may underlie the evolution of cancers. Wigler's group focuses on breast cancer and leukemias, and they are engaged in clinical studies with major research hospitals to discover mutation patterns predicting treatment response and outcome. In collaboration with Jim Hicks and Gregory Hannon at CSHL, the lab has applied microarray techniques and hybrid selection to explore the role of epigenetics in cancer. With Jonathan Sebat, they have made headway in the discovery of the causative mutations in autism. Their results show that spontaneous mutation has a far greater role in autism than previously suspected. They have developed a new theory of autism's genetic basis that explains otherwise bewildering patterns of inheritance and are testing the new genetic model in other disabling genetic

disorders. Wigler also has spearheaded the development of the Center for Quantitative Biology at CSHL, with initial funding from the Simons and Starr foundations.

Michael Zhang's laboratory develops mathematical and computational methods that can be combined with advanced experimental technologies to transform data into biological knowledge about transcription and gene expression, work that has manifold implications for the study of cancer and many other diseases. Their tools, used by investigators throughout CSHL and beyond, are designed to identify functional genetic elements within molecular sequences as well as pathways that control and regulate gene expression. Zhang's group has developed a series of computational tools that make use of statistical pattern-recognition techniques to identify exons, promoters, and posttranslational modification signals in large genomic DNA sequences. They also study alternative splicing of exons and collaborate with other labs to characterize splicing enhancers and silencers. This past year, Zhang's team published widely cited studies on optimization of sequencing with next-generation Solexa equipment, using software to predict promoter regions at high resolution, and, in collaboration with Adrian Krainer at CSHL, defining the regulatory networks of two related tissue-specific (brain and muscle) splicing factors, Fox-1 and Fox-2.

Rapid growth of the research literature in genomics and molecular biology threatens the ability of scientists to digest and make use of valuable data. CSHL Fellow Ivan Iossifov seeks to develop automated methods for extracting, structuring, and interpreting research data. Building on text-mining methods that he developed in his Ph.D. work, Iossifov is now focusing on using these to build representations of molecular networks for humans and for two model organisms, *Mus musculus* (lab mouse) and *Saccharomyces cerevisiae* (baker's yeast). In collaboration with Partha Mitra at CSHL, he is also working on a means of organizing information about connections among brain regions, work that could prove valuable in efforts to trace the etiology of common neurodevelopmental disorders such as autism, schizophrenia, and bipolar disorder.

INTEGRATIVE SYSTEMS NEUROBIOLOGY

P. Mitra P. Andrews V. Pinskiy
J. Bohland D. Valente
H. Bokil H. Wang
J. Kulkarni C. Wu

Our basic research philosophy is to combine theoretical, computational, and experimental approaches for the study of complex biological systems, with a particular focus on brain function. Efforts in our group fall into three main areas: informatics, theory, and experimental work.

Our neuroinformatics research involves the application of analytical tools to large volumes of neurobiological data as well as the development of informatics infrastructure for data and knowledge integration. Our study in the area of theoretical engineering applies theories developed in human-engineered systems to study the theoretical principles underlying biological systems. The final area of our research is experimental and consists of collaborative studies in multiple species including *Drosophila*, zebra finch, macaque monkey, and human infants.

Continuing in our laboratory in 2008 were Peter Andrews (scientific informatics manager), Hemant Bokil (postdoctoral fellow), Haibin Wang (postdoctoral fellow), Dan Valente (postdoctoral fellow), Jason Bohland (scientific informatics manager), and Caizhi Wu (postdoctoral fellow). Vadim Pinskiy (graduate student) and Jayant Kulkarni (postdoctoral fellow) joined us this past year, and John Lin (scientific informatics developer) left to work in industry.

We also have close collaborative ties with multiple research groups at CSHL and other institutions, which currently include Dr. Nicholas D. Schiff at the Weill Medical School of Cornell University, where Dr. Mitra is an adjunct associate professor; a consortium of zebra finch researchers including Ofer Tchernichovski at City College of New York (CCNY); Josh Dubnau at CSHL to perform an integrative analysis of memory formation in *Drosophila*; the Brain Architecture Project, with collaborators at multiple institutions, notably Larry Swanson at the University of Southern California and Michael Hawrylycz at the Allen Institute of Brain Research; and a recently initiated collaboration with Gregory Hannon at CSHL on improving next-generation sequencing technologies.

The Brain Architecture Project

J. Bohland, H. Bokil, C. Wu

The broad goal of the Brain Architecture Project is to characterize human brain architecture, in particular to describe the *connectivity matrix* across areas in the brain. Project objectives include extracting knowledge about human brain connectivity from the extant literature, placing these data in a structured database system, and developing tools for analysis and visualization of brain architecture.

Before the 2009 Meeting of the Society for Neuroscience, we launched the official project website at http://brainarchitecture.org. The website currently contains several key components: (1) access to a database containing information about neuroanatomical connectivity reported in the *human* brain, (2) a tool that enables comparison of different anatomical atlases of the human brain, and (3) two tools for searching the full text of a large corpus (>50,000 papers) of scientific papers related to brain architecture using a combination of keyword or semantic queries.

We have also formed collaborations with Dr. Pavel Osten (CSHL) and Dr. Harvey Karten (University of California, San Diego). We have proposed launching a connectivity project for the mouse at CSHL by developing two copies of a semiautomated experimental and data analysis *pipeline* to be used in high-throughput efforts to generate the *first* comprehensive circuit diagram for the mouse, a species of high importance due to its prevalent use in basic and clinical research. The proposal calls for using both "conventional" neuroanatomical tracers and engineered viral vectors to trace projection patterns from 400 injection sites in triplicate.

The Brain Architecture Project hosted a meeting at the CSHL Banbury Center in May 2008 entitled The Architectural Logic of Mammalian CNS. At this meeting, participants discussed our current understanding of the basic large-scale organization of brain circuits in vertebrate animals. The Brain Architecture Project team

presented our ongoing work and progress in the project and received important guidance from many experts in neuroanatomy and informatics. An additional major focus of the meeting was a discussion of a community-driven proposal for systematically determining the *mesoscale* connectivity map in model organisms, starting with the mouse brain.

An additional aspect of brain organization of great interest is its *genetic architecture*, i.e., in what brain areas/cell groups are specific genes expressed, and how does the organization of gene expression relate to other aspects of brain organization?

We have established a relationship with researchers from the Allen Institute for Brain Science to analyze spatial gene expression patterns in the Allen Brain Atlas (ABA). The ABA consists of genome-wide expression maps for the adult mouse brain, including raw high-resolution images of in situ hybridization sections and smoothed and coregistered expression volumes for each gene. We have performed a large-scale analysis of a set of expression volumes for ~3000 genes. In one aspect of this analysis, we used clustering techniques to segment the mouse brain based on the similarity of the pattern of expression across this gene set at each voxel (spatial location). The results, illustrated in Figure 1, indicate that the segments that arise from clustering based on gene expression largely align with an atlas derived from classical neuroanatomy (e.g., by looking at cell types and cell densities). Note, for example, the division of the mouse cortex into clusters aligned to different lamina. Other aspects of our analyses have focused on the localization of gene expression and, for diseases with known genetic etiology, the relationship between expression patterns of implicated genes and the brain areas in which the disease causes pathological changes.

Toward an Integrative Model of Memory in *Drosophila*

D. Valente

During the past year, we have continued the development of a learning assay that will allow us to probe each level of biological organization, during the learning process, from genetics to behavior. This assay, the *Drosophila* flight simulator, has been used in numerous studies to show that flies can be conditioned to avoid certain patterns presented to them in their visual environment. After numerous attempts at reproducing the published results were unsuccessful, we performed a series of experiments aimed at addressing the major differences between our setup and those described in the literature. Although further investigation into these differences has left the source of the discrepancy undetermined, it has been essential in elucidating the behavior

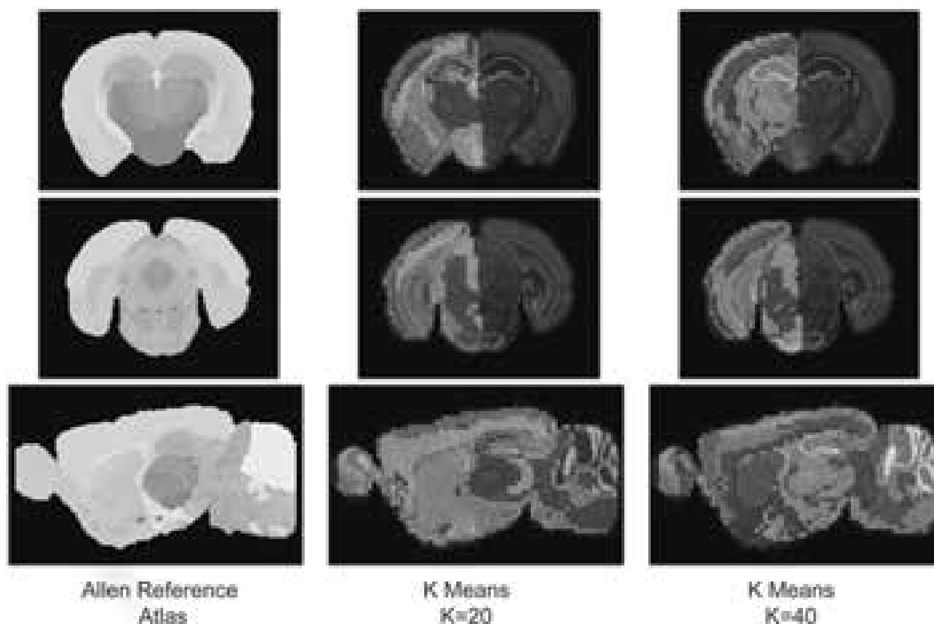

FIGURE 1 Segmentation of mouse brain based on the expression patterns of 3000 genes (*center* and *right columns*) results in clusters that largely align with classical neuroanatomical regions.

FIGURE 2 A fruit fly in the flight simulator.

of flies in this paradigm. Furthermore, we have used data obtained from classical and operant conditioning experiments to develop a more complete quantitative phenotype for flies in these assays, and we continue to develop new measures and metrics to assess performance in this paradigm. Most notably, we have shown the unconditioned response of flies to the aversive stimulus on millisecond timescales—a necessary step in understanding the sensorimotor integration required to create an association during learning.

In addition to the studies of flies in flight, we have continued the development of better methods for quantitative phenotyping of the locomotor and exploratory behavior of flies in the open field. In a collaboration with Ilan Golani of Tel Aviv University, we have developed mathematical techniques to describe the effect of cocaine and alcohol on initiation of movement and the effect of the drugs on movement behaviors. From this research, two papers are currently being prepared for publication. Furthermore, we worked on a collaboration with François Bolduc at the University of Alberta (formerly of Tim Tully's lab here at CSHL) using similar phenotypic measures to study social interaction defects in flies mutant for the fragile-X mental retardation gene (*Fmr1*) as a model for social defects in autistic patients. This also resulted in a paper that is currently in the submission process.

Finally, we continued our collaboration with Josh Dubnau's lab on the project "Experimental evolution of *rutabaga* suppression: Modeling gene epistasis underlying behavior." Our contribution to this project has been a series of calculations important for the experimental design, the creation of web tools for analyzing and storing behavioral results, as well as analysis of genotypic results to determine the elevation or suppression of allelic frequencies in response to selection.

Alta-Cyclic: A Self-optimizing Base Caller for Next-generation Sequencing

Y. Erlich [in collaboration with the Hannon lab, Cold Spring Harbor Laboratory]

This project sought to improve the base-calling procedure for Illumina Genome Analyzers to obtain more accurate and longer sequence reads. Such an improvement would boost overall output per run, increase genomic coverage, and improve the ability to detect sequence variants. Longer reads also increase mapping precision and may even enable de novo genome assembly.

Inspired by ideas from communication theory, we analyzed the sequencing platform's nonstationary distortion factors, because these accumulate throughout the run and reduce accuracy in later sequencing cycles. We developed a novel base caller named Alta-Cyclic, which is designed to address these distortions. Alta-Cyclic works in two stages: the training stage and the base-calling stage. During the training stage, Alta-Cyclic learns run-specific noise patterns according to our model and finds an optimized solution that reduces the effect of these noise sources. The optimization is mainly achieved by supervised learning using a rich DNA library with a known reference genome. Alta-Cyclic then enters the base-calling stage and reports all of the sequences from the run with the optimized parameters.

Benchmarking Alta-Cyclic with respect to the Illumina base caller after a long run of 78 cycles revealed a substantial increase in the number of correct base calls. For instance, in the case of a phi-X library, Alta-Cyclic reported more than fourfold, 78-nucleotide-long, fully correct reads, which comprised 22% of the total sequences. For comparison, The Illumina base caller reported only 5% of the total sequences without any misdetection.

Integrative Study of Zebra Finch Vocal Development

H. Wang

Our principal goals for this study are to provide the informatics infrastructure for data management and mining, develop signal processing algorithms, and analyze experimental data from collaborating laboratories.

We applied statistical measures to the analysis of zebra finch song from an experimental study of evolution of song culture in a laboratory setting (in collaboration with the Tchernichovski lab at City College of New York). In a recursive one-to-one training experi-

ment, an isolated adult male was used to train a young zebra finch, which after maturity, trained yet another young bird, and so on. We found that the songs of the isolate tutor (ISO) were inherited (imitated) along the training lineage, but, at the same time, they were gradually and steadily transformed toward wild-type-like songs in a few generations. In the second experiment, a seminatural island colony of zebra finch was established from an isolated male and three females. We were able to observe and characterize the emergence and evolution of song culture, using the same analysis methods.

We proposed a theoretical framework to understand how the song culture evolves through interactions among the phenotype, the genotype, and the environment. In analogy to the parental-effect model in quantitative genetics, we partitioned the phenotypic value into genotypic and environmental components and assumed an additive model for genetic variance. Our model illustrates that the song culture results from an extended developmental process, a "multigenerational" phenotype partly genetically encoded in an isolated founding population and partly in environmental variables, but taking multiple generations to emerge.

It has long been realized that a dimensionally accurate and detailed atlas of the zebra finch brain is an essential tool. Recent progress in neuroanatomical research has made such a digital atlas possible, driven by the availability of automated scanning microscopes and the ability to store and analyze tera/petabyte-scale data sets. Through a collaboration with Harvey Karten at the University of California, San Diego, we have obtained high-resolution images of Nissl- and myelin-stained zebra finch brain sections that are available at www.zebrafinch.org/atlas as part of our informatics infrastructure for zebra finch birdsong study.

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COPY-NUMBER ANALYSIS AND HUMAN DISEASE

M. Wigler G. Alex, C. Algieri, S. Chakraborty, K. Cook, Y. Eberling, D. Esposito, V. Grubor, J. Hicks, L. Hufnagel, R. Kandasamy, J. Katz, J. Kendall, A. Krasnitz, Y.-H. Lee, A. Leotta, D. Levy, S. Marks, J. Meth, L. Muthuswamy, N. Navin, D. Pai, M. Riggs, L. Rodgers, M. Ronemus, J. Troge, B. Yamrom

MAMMALIAN GENETICS

We study variations in the human genome and their association with disease and genetic disorders. The variations we study arise when a large segment of the genome is duplicated or deleted. Such copy-number variations, or CNVs, can arise somatically or in the germ line. Somatic variations are often seen in cancer, and they distinguish cancer cells from the normal cells of the body. They provide clues for the origin and behavior of the cancer and possibly its early detection. Germline variations distinguish individuals from one another and may be inherited, in which case, they are known as copy-number polymorphisms, or CNPs, or they may arise spontaneously, in which case, they serve as engines of human diversity or can cause devastating genetic disorders, such as autism.

Part of our lab uses copy-number data and DNA-methylation status to study solid cancers, especially breast cancer (Hicks et al., *Genome Res. 16:* 1465 [2006]) and B-cell chronic leukemia (Grubor et al. 2008). We seek to identify the genes most frequently mutated in cancers and leukemias and to determine whether genomic data can be used to predict the outcome of the disease, its response to therapy, and the early detection of its recurrence. Another part of our lab studies copy-number variation that may underlie autism, congenital heart defects, and other profound disorders of normal human development. A large portion of the lab is composed of quantitative/computational scientists who work on methods for data processing, visualization, and interpretation.

Until recently, our studies were largely based on a high-throughput, high-resolution, microarray technology developed in our lab called representational oligonucleotide microarray analysis (ROMA) (Lucito et al., *Genome Res. 12:* 2291 [2003]), which itself was based on an earlier technology developed in our lab called representational difference analysis (RDA) (Lisitsyn et al., *Science 259:* 946 [1993]). RDA was previously used to discover tumor suppressors, oncogenes, and pathogens. ROMA is one form of a more general technology called comparative genome hybridization, or CGH. Methodology is constantly evolving, and part of our laboratory works on an extension to ROMA called methylation detection oligonucleotide microarray analysis (MOMA) used to study DNA methylation. We still use ROMA, for example, when the amount of a sample is limiting, but our CGH work is now mainly based on commercial platforms that have higher resolution than ROMA. We have recently been investigating the applications of high-throughput, single-molecule, DNA sequencing to discover and detect copy-number variation, even at the level of single cells.

CANCER AND LEUKEMIA

Our work in cancer applies genome analysis to both basic and clinical problems. This year, we continued our clinical studies on breast cancer, with an emphasis on marker discovery and detection, that we hope will directly benefit patient care. We participate with the Memorial Sloan-Kettering Cancer Center and Yale University in the retrospective genomic analysis of breast cancer cases from previous clinical trials designed to discover better markers for the successful response to chemotherapeutic drugs. Our present work suggests the superiority of CGH to fluorescent in situ hybridization (FISH) for the evaluation of responsiveness to Herceptin. We also have continued our collaboration with the Radium Hospital in Oslo, Norway, in clinical trials initiated to understand the role of bone marrow micrometastasis in the clinical outcome of breast cancer. We use both copy-number and DNA methylation to find markers for survival.

We continue our efforts to find cancer genes in breast cancer, in collaboration with two Scandinavian centers, led by Anders Zetterberg at the Karolinska Institute, Sweden, and Anne-Lisa Borresen-Dale at the Radium Hospital, Norway, as well as Cliff Huddis and Larry Norton at the Memorial Sloan-Kettering Cancer Center (MSKCC), New York. These studies have elucidated a set of loci, called epicenters, that are the recurrent sites for genome amplification and deletion in breast cancer. We and many others presume that the epicenters are

locations where the genes that drive the progression of malignancy reside. Hence, the elucidation of these loci may facilitate less-expensive tools for the evaluation of the clinical state of cancers and ultimately to gene discovery.

We have continued our studies on tumor heterogeneity. Clues to the progression of cancer, and its clinical profile, are missed if we assume that each individual's cancer is a single clone with a single molecular history. Work in progress indicates that at least half of breast cancers are composed of multiple clones. Our work proves that sampling one region of the cancer for molecular markers will often yield an incomplete picture of the state of the cancer. The careful analysis of clonal variety should yield insight into breast cancer: the order and mechanism by which it evolves and even if it has already metastasized.

Our studies on tumor heterogeneity have motivated us to attempt analysis of the genome of single cancer cells. Preliminary studies suggest that it is technically feasible to sequence the DNA of single cells. We use the DNA sequence reads as "counters" to determine the amplification/deletion pattern in the genome. We are preparing to use this method to detect the presence of cancer cells in biopsies, which could eventually lead to clinical tests for cancer recurrence and risk assessment.

In our studies of B-cell chronic leukemia (CLL), a collaboration with Nick Chiorazzi of North Shore University Hospital, Manhasset, New York, we have used ROMA to identify essentially all of the known recurrent lesions observed in that disease and discovered several new epicenters in addition. As with breast cancer, we now have evidence of multiple clones of common origin in leukemia from the same patient. Using high-resolution microarrays, we also have found preliminary evidence for a set of signature genomic events in CLL at a set of transcriptional regulator genes. We are presently investigating whether these signature events are caused by deletion or are in some way artifacts of chromatin restructuring specific to CLL.

We have made significant progress in our studies of DNA methylation in cancer. We previously described a microarray platform (MOMA) designed to detect methylation of CpG islands (Kamalakaran et al., *Nucleic Acids Res.*, in press). In our studies of samples supplied from the Radium Hospital in Norway, we have identified CpG-rich islands that change methylation state during the initiation and progression of breast cancer. The genome-wide methylation patterns clearly distinguish normal tissue and tumor tissue and might be useful for the analytic deconvolution of complex tissue mixtures into their cellular components. The methylation patterns cluster into subgroups that correlate with genome and expression profile clustering. In collaborations with Greg Hannon and Dick McCombie at CSHL, we have used DNA sequencing to demonstrate that there are DNA methylation switch zones. Detection of methylation switches in these zones may facilitate cancer detection and clinical subtyping.

GENETIC DISORDERS

After our discovery that copy-number variation is common in the human gene pool (Sebat et al., *Science 305:* 525 [2004]), we studied the role of CNVs in human disease and, in particular, in the role of spontaneous (or de novo) CNVs in the germ line. Our findings established that germ-line mutation was a more significant risk factor for autism spectrum disorders than previously recognized (Sebat et al., *Science 316:* 445 [2007]) and established a new approach for the further study of the genetic basis of this and other genetic disorders, such as schizophrenia and congenital heart disease.

One of the de novo events we identified was a deletion on 16p. This event has now been shown by two other groups to explain perhaps as much as 1% of autism. We assisted Alea Mills at CSHL to engineer mice with the orthologous deletion on mouse chromosome 7. We are hopeful that these mice will provide animal models suitable for understanding the underlying neuropathology of the condition and the search for palliative treatments.

Analysis of autism incidence in families, a collaboration with Kenny Ye at Albert Einstein School of Medicine, provided evidence for a unified theory of the genetic basis for the disorder (Zhao et al., *Proc. Natl. Acad. Sci. 104:* 12831 [2007]). Autism families are divided into simplex (only one affected child) and multiplex (multiply affected children). By inspecting the records from the AGRE consortium, we found that the risk to a male newborn in an established multiplex family is nearly 50%, the frequency expected of a dominant disorder. We compute that autism incidence and sibling concurrence rates are consistent with a model in which new mutations with a strong contribution arise sufficiently frequently to explain the majority of autism and are passed from a resistant carrier parent to a child in a dominant fashion. One major source of resistance is gender. Autism has a much lower incidence in females.

We are now in the midst of a larger study of spontaneous mutation in autism, based on a population of simplex families collected by the Simons Foundation. Early initial results confirm our previous findings, and we

observe de novo mutation more frequently in children with autism than in their unaffected siblings. Because our new studies are performed with higher-resolution microarrays, we also see many more examples of narrow new mutations (altering only a few genes), thus expanding our list of good candidate genes involved in the disorder. There is a male bias to the detection of narrow mutations, but we see little gender bias to detecting broad mutations (altering many genes). Because there should be no gender bias in the incidence of new mutation, the detection biases suggest to us that at least two contributory genes are targeted in the broad lesions, equalizing susceptibility in males and females. One clear benefit of our large study will be in the design of clinical tests for genetic counseling and early detection. Eventually, this knowledge will contribute to finding and matching patients with the most effective therapies.

DATA GENERATION AND ANALYSIS

The major part of our group's effort centers on the generation, analysis, and interpretation of high-volume data. This entails developing protocols for conducting microarray experiments, determining quality control, probe evaluation, signal extraction, and segmentation (the method of "observing" copy-number variation); comparisons of sets of experiments, including new statistical measures, data reduction, and data summary; and construction of databases so that we can communicate our results to other investigators. Our novel contributions include methods to attenuate system noise in array hybridizations, parametrize hybridization performance, detect and correctly call regions of genetic polymorphism, detect de novo events, classify cancers for outcome, and define the epicenters of genetic change in cancers and leukemias.

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K. Cook

COMPUTATIONAL GENOMICS

M.Q. Zhang	N. Dimitrova	W. Luo	X. Wang
	E. Domb	M. Paradesi	Z. Xuan
	D. Lewis	J. Rosenfeld	C. Xue
	W. Liao	A.D. Smith	C. Zhang

In the last year, Chao Lin finished his Ph.D. and went on to a postdoctoral fellowship at The Rockefeller University. Xiaowo Wang took an assistant professor position at Tsinghua University in China, and Dr. Andrew Smith took a tenure-track faculty position at the University of Southern California. Eric Domb was our URP student from Princeton University and Will Liao was our rotation student from Stony Brook University. Dr. Zhenyu Xuan has been promoted to Research Assistant Professor and is heading the Bioinformatics Resource Service Core Facility of the Cancer Center with Dr. Weijun Luo and Mr. Martin Paradesi on staff. 2008 has been a very productive year, despite the severe budget cuts from the government.

COMBINATORIAL PATTERNS OF HISTONE ACETYLATIONS AND METHYLATIONS IN THE HUMAN GENOME

Histones are characterized by numerous posttranslational modifications that influence gene transcription. However, because of the lack of global distribution data in higher eukaryotic systems, the extent to which gene-specific combinatorial patterns of histone modifications exist remains to be determined. In collaboration with the Zhao Lab at NHLBI/NIH, we studied the combinatorial patterns derived from the analysis of 39 histone modifications in human CD4(+) T cells. Our results indicate that a large number of patterns are associated with promoters and enhancers. In particular, we identified a common modification module consisting of 17 modifications detected at 3286 promoters. These modifications tend to colocalize in the genome and correlate with one another at an individual nucleosome level. Genes associated with this module tend to have higher expression, and addition of more modifications to this module is associated with further increased expression. Our data suggest that these histone modifications may act cooperatively to prepare chromatin for transcriptional activation. This is the first comprehensive chromatin immunoprecipitation sequencing (ChIP-seq) mapping of the human epigenome at the nucleosome resolution (Wang et al. 2008).

DEFINING THE REGULATORY NETWORK OF THE TISSUE-SPECIFIC SPLICING FACTORS FOX-1 AND FOX-2

The precise regulation of many alternative splicing (AS) events by specific splicing factors is essential to determine tissue types and developmental stages. However, the molecular basis of tissue-specific AS regulation and the properties of splicing regulatory networks (SRNs) are poorly understood. In collaboration with the Krainer lab here at CSHL, we comprehensively predicted the targets of the brain- and muscle-specific splicing factor Fox-1 (A2BP1) and its paralog Fox-2 (RBM9) and systematically defined the corresponding SRNs genome-wide. Fox-1/2 factors are conserved from worm to humans, and they specifically recognize the RNA element UGCAUG. We integrated Fox-1/2-binding specificity with phylogenetic conservation, splicing microarray data, and additional computational and experimental characterization. We predicted thousands of Fox-1/2 targets with conserved binding sites, at a false discovery rate (FDR) of ~24%, including many validated experimentally, suggesting a surprisingly extensive SRN. The preferred position of the binding sites differs according to the AS pattern and determines either activation or repression of exon recognition by Fox-1/2. Many predicted targets are important for neuromuscular functions and have been implicated in several genetic diseases. We also identified instances of binding-site creation or loss in different vertebrate lineages and human populations, which likely reflect fine-tuning of gene expression regulation during evolution (Zhang et al. 2008).

LARGE-SCALE NETWORK-BASED PREDICTION OF HUMAN DISEASE GENES

Deciphering the genetic basis of human diseases is an important goal of biomedical research. On the basis of the assumption that phenotypically similar diseases are caused by functionally related genes, we propose a computational framework that integrates human protein–protein interactions, disease phenotype similarities, and known gene–phenotype associations to capture the complex relationships between phenotypes and genotypes.

In collaboration with Tsinghua University, we developed a computational tool named *CIPHER* to predict and prioritize disease genes, and we showed that the global concordance between the human protein network and the phenotype network reliably predicts disease genes. Our method is applicable to genetically uncharacterized phenotypes, effective in the genome-wide scan of disease genes, and is also extendable to explore gene cooperativity in complex diseases. The predicted genetic landscape of more than 1000 human phenotypes reveals the global modular organization of phenotype–genotype relationships. The genome-wide prioritization of candidate genes for more than 5000 human phenotypes, including those with undercharacterized disease loci or even those lacking known association, is publicly released to facilitate future discovery of disease genes (Wu et al. 2008). This work was highlighted by a *Nature China* article and was ranked number one in citations among *Molecular Systems Biology* published papers in the category.

DISEASE GENE REGULATION

In collaboration with the Nahle lab at Washington University, we studied CD36-dependent regulation of muscle FoxO1 and PDK4 in the peroxisome proliferation-activated receptor (PPAR) δ/β-mediated adaptation to metabolic stress. The transcription factor FoxO1 contributes to the metabolic adaptation to fasting by suppressing muscle oxidation of glucose, sparing it for glucose-dependent tissues. Our findings suggest that CD36-dependent fatty acid activation of PPAR δ/β results in the transcriptional regulation of FoxO1 as well as PDK4, recently shown to be a direct PPAR δ/β target. FoxO1 in turn can regulate CD36, lipoprotein lipase, and PDK4, reinforcing the action of PPAR δ/β to increase muscle reliance on fatty acid. The findings could have implications in the chronic abnormalities of fatty acid metabolism associated with obesity and diabetes (Nahle et al. 2008). Recently, we also studied the regulation of the PDK4 isozyme by the Rb-E2F1 complex. Loss of the transcription factor E2F1 elicits a complex metabolic phenotype in mice underscored by reduced adiposity and protection from high-fat diet-induced diabetes. Our analysis illustrates how the E2F1 mitogen directly regulates PDK4 levels and influences cellular bioenergetics, namely, mitochondrial glucose oxidation. These results are relevant to the pathophysiology of chronic diseases such as obesity and diabetes, where PDK4 is dysregulated and could have implications pertinent to the etiology of tumor metabolism, especially in cancers with Rb pathway defects (Hsieh et al. 2008).

TRANSCRIPTIONAL NETWORK IN BREAST CANCER

In collaboration with the Akiyama and Aburatani labs at the University of Tokyo, we completed an integrative bioinformatics analysis of transcriptional regulatory programs in breast cancer cells. To gain insights into the transcriptional programs that drive breast cancer tumor progression, we integrated regulatory sequence data and expression profiles of breast cancer into a Bayesian Network and searched for *cis*-regulatory motifs statistically associated with given histological grades and prognosis. Our analysis found that motifs bound by ELK1, E2F, NRF1, and NFY are potential regulatory motifs that positively correlate with malignant progression of breast cancer.

The results suggest that these four motifs are principal regulatory motifs driving malignant progression of breast cancer. Our method offers a more concise description of transcriptome diversity among breast tumors with different clinical phenotypes (Niida et al. 2008).

MAPPING THE NEXT-GENERATION SEQUENCING READS

Next-generation sequencing has the potential to revolutionize genomics and impact all areas of biomedical science. New technologies will make resequencing widely available for such applications as identifying genome variations or interrogating the oligonucleotide content of a large sample (e.g., ChIP-seq). The increase in speed, sensitivity, and availability of sequencing technology brings demand for advances in computational technology to perform associated analysis tasks. The Solexa/ Illumina 1G sequencer can produce tens of millions of reads, ranging in length from ~25 to 50 nucleotides, in a single experiment. Accurately mapping the reads back to a reference genome is a critical task in almost all applications. Two sources of information that are often ignored when mapping reads from the Solexa technology are the 3′ ends of longer reads, which contain a much higher frequency of sequencing errors, and the base-call quality scores. To investigate whether these sources of information can be used to improve accuracy when mapping reads, we developed the *RMAP* tool, which can map reads having a wide range of lengths and allows base-call quality scores to determine which positions in each read are more important when mapping. We applied *RMAP* to analyze data resequenced from two human bacterial artificial chromosome (BAC) regions for varying read lengths and varying criteria for use of quality scores. *RMAP* is freely available for downloading at http://rulai.cshl.edu/rmap/.

Our results indicate that significant gains in Solexa read-mapping performance can be achieved by considering the information in the 3′ ends of longer reads and appropriately using the base-call quality scores. The *RMAP* tool will enable researchers to effectively exploit this information in targeted resequencing projects (Smith et al. 2008). To further improve speed and sensitivity, we have collaborated with the Li lab at the University of Waterloo, Canada, to develop a novel spaced seed-based algorithm called *ZOOM* that can be used for any platforms of sequencing technologies, including double-end sequencing reads. It also allows gaps in read mapping. Using this framework, we are able to map the Illumina/Solexa reads of 15x coverage of a human genome to the reference human genome in 1 CPU day, allowing two mismatches, at full sensitivity. The software is freely available to noncommercial users at http://www.bioinfor.com/zoom.

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In Press

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PLANT BIOLOGY

Plant genetics has long had a major role in the advance of all areas of research at CSHL, epitomized by the work of Nobel Laureate Barbara McClintock, who discovered transposable genetic elements—which she called "jumping genes"—in the 1940s. The contemporary effort to understand the genetic and cell biological mechanisms that enable plants to grow and develop has obvious implications not only for agriculture, but also for human health, as well as the development of next-generation biofuels and related alternative energy sources. Plant geneticists at CSHL have been among the leaders of efforts to sequence the first plant genomes, of *Arabidopsis* and maize, and continue to be at the center of sequencing and genome-annotation projects involving a host of cereal crops that feed the growing population of our planet. CSHL scientists have been among the pioneers in the study of RNAi, a gene-regulatory mechanism unknown only a decade ago, which has been shown, first in plants, then in other organisms, to have a vital and evolutionarily ancient genome-defending role.

David Jackson and colleagues study genes and signals that regulate plant growth and architecture. This year, they built on past success in identifying a gene that controls communication between plant cells via small channels called plasmodesmata. These channels, which direct the flow of nutrients and signals through growing tissues, are regulated during development. Jackson's team performed a genetic screen and found some mutants that are affected in transport, one of which was found to affect redox signaling. This suggests a mechanism through which plant cells can adjust trafficking in the channels in different developmental stages. The lab also cloned a gene called *tasselsheath*, whose activity they correlated with the shutting down of leaf growth when flowering gets under way. Jackson's past success in developing fluorescent-protein-tagged maize lines bore fruit when a team that included Jackson this year identified a gene called *sparse inflorescence1* that proves to be vital in the formation of corn "ears."

Zachary Lippman's research focuses on a universal growth habit in plants called sympodial growth, seen in widely varying species from orchids to tomatoes to trees. It is characterized by a renewal of growth that follows a cessation of growth following prior flowering, resulting in an array of stems. Lippman's lab seeks to understand how sympodial growth affects the vigor and reproductive success of a plant, specifically its ability to produce vegetative shoots and flower-bearing shoots called inflorescences. In the tomato plant and other members of the plant family to which it belongs, called *Solanaceae,* they study genes that control the branching of flowering shoots and how those genes function to control flower production. Their aim is to decipher the molecular basis of the natural variation for inflorescence branching seen in that important family, with an eye to increasing crop yields.

Epigenetic mechanisms of gene regulation—chemical and conformational changes to chromatin bundles of DNA and protein—have important impact on genome organization and inheritance and on cell fate. These mechanisms are conserved in eukaryotes and provide an additional layer of information superimposed on the genetic code. Robert Martienssen, a pioneer in the study of epigenetics, investigates mechanisms involved in gene regulation and stem cell fate in yeast and model plants including *Arabidopsis* and maize. He and colleagues have shed light on a phenomenon called position-effect variegation, associated with plant color diversity and caused by inactivation of a gene positioned near densely packed chromosomal material called heterochromatin. They have found that heterochromatin is programmed by small RNA molecules arising from repeating genetic sequences. This past year, they addressed a nagging conundrum: Because genes contained within heterochromatin are silenced, how can they give rise to RNA molecules that help to modify the histone proteins around which DNA is spooled? They solved the puzzle by tracking cells through their cycle of growth and division. They also mapped changing epigenetic modifications enabling mobile genetic elements to run amok within a genome.

The growing tips of plants, called meristems, contain a population of stem cells that serve as a persistent source of daughter cells from which new organs arise. They also produce signals important for the determination and patterning of lateral organs. Marja Timmermans and colleagues are using a

genomic approach to study genes active in the meristem. They have used mutational analyses to identify a protein complex that suppresses stem cell fate during organ development. This complex includes the chromatin remodeling factor HIRA, an epigenetic regulator that helps to control stem cell identity. This year, the lab made progress in elucidating the molecular mechanism through which HIRA acts, demonstrating its role in specifying stem cell niches during embryogenesis and, later on, after commitment to differentiation, in shutting off stem cells. They also identified regulatory mechanisms that allow for the precise spatial accumulation of developmentally important small RNAs in plants. This work revealed that polarity in leaves is established via mobile small RNAs; a description of the mobile small RNA, tasiR-ARF, in pattern formation suggests possible roles of other small RNAs as morphogen-like signals during development in plants and animals.

DEVELOPMENTAL BIOLOGY: STEM CELLS, SIGNALING, AND CONTROL OF PLANT ARCHITECTURE

D. Jackson Y. Benitez, P. Bommert, K. Chen, S. Choi, S. DeBlasio, A. Goldshmidt, E. Jimenez, R. Johnston, Y.K. Lee, M. Pautler, N. Satoh-Nagasawa, M. Scricco, J. Wang, C. Whipple, X.M. Xu, J. Yuan, T. Zadrozny

Research in our lab is aimed at identifying novel genes, signals, and pathways that regulate multicellular growth and development. All organisms develop by carefully controlling the flow of information ("signals") that passes between cells and tissues. We are particularly interested in discovering these signals and finding out how they are transmitted and how they function. As an example, we have identified genes that control how plant cells communicate with one another through small channels, called plasmodesmata (PD). These channels were described in plants more than 100 years ago, but their central importance and how they are regulated are just beginning to emerge. Plasmodesmata are critical for plant growth because they allow the flow of nutrients and signals through growing tissues. In the past year, we discovered an important mechanism by which transport through these channels is regulated via redox signaling, and we have also found a novel chaperone gene that regulates the specific cell-to-cell transport of KNOTTED1, a transcription factor. KNOTTED1 is a homeodomain protein that is expressed in plant stem cells and is necessary to keep these cells in a pluripotent state.

We also continue to identify maize genes with novel roles in shoot development and plant architecture. We have recently identified genes that control branching, stem cell proliferation, and leaf growth. One of these, *tasselsheath1*, encodes a transcription factor that appears to have evolved a novel function in the grasses. This gene is particularly interesting because leaf suppression in the inflorescence is an important crop yield trait. Finally, we continue to develop a collection of "fluorescent-protein"-tagged maize lines that are an essential resource for all maize researchers. This is the first collection of its kind and promises to enhance maize genetics research through characterization of developmental gene pathways and identification of maize promoters that are useful tools for basic research as well as in crop improvement.

Control of Intercellular Trafficking in *Arabidopsis thaliana*

Y. Benitez

Intercellular communication is essential for plant development and environmental adaptability. PD—channels embedded in the cell wall—connect plant cells and enable the transport of metabolites, viral and endogenous proteins, and RNAs. Recently, we identified a factor that regulates this transport by a genetic screen in the model plant *Arabidopsis thaliana*. We used green fluorescent protein (GFP) as a reporter to isolate mutant plants affected in PD transport. GFP moves freely between plant cells in developing plant tissues, and we screened for mutants blocked in this transport. Five mutants were identified and named *gat* (pronounced "gate") for "*GFP-arrested trafficking.*" Severe restriction in transport was found in four of the mutants (*gat1, 2, 4,* and *5*). In contrast, *gat3* mutants showed a milder block in the transport. These differences in GFP mobilities were also reflected in the seedling phenotypes of the mutants: *gat1, 2, 4,* and *5* arrested early after germination and developed only a short root and small leaf primordia, but *gat3* seedlings did not show any obvious developmental defects.

Characterization of *gat1* mutants revealed that they were defective in a thioredoxin gene, *TRX-m3*, involved in cellular redox responses. Characterization of the mutant and ectopic expression phenotypes suggested that GAT1 regulates communication in the meristem (where stem cells are located) and other immature tissues by controlling the production of the polymer callose. Callose deposition in PD is a normal mechanism to close down these channels during development of specific cell types that need to be isolated. Analysis of the redox status of the mutant and the phenotype of wild-type plants grown in oxidants suggests that this regulation is dependent on the generation of reactive oxygen species within the cell.

Other trafficking mutants identified in this screen (*gat2*, *4,* and *5*) also showed hyperaccumulation of reactive oxygen species and callose in the root meristem, suggesting that these mutants, together with *gat1*, are components of a redox-dependent mechanism that regulates callose deposition in the PD. The identification of the corresponding genes will provide new insights in this mechanism.

In contrast, *gat3* seems to be part of a separate mechanism in PD regulation. *gat3* mutants behave in a dominant manner and develop normally, although GFP movement is affected throughout development. We are now working to identify the *GAT3* gene, which will shed light on a potentially new mechanism for PD regulation.

A Chaperonin Regulates KNOTTED1 Cell-to-Cell Trafficking

X.M. Xu, J. Wang, M. Scricco

Cell-to-cell communication has critical roles in specifying cell fate and coordinating development in all multicellular organisms. A new paradigm for such communication in plants is the selective trafficking of informational macromolecules, e.g., transcription factors through PDs, channels that traverse the cell wall and connect all plant cells.

In addition to cell-fate specification, PDs are also involved in viral movement, transport of metabolites, and cell-to-cell spread of RNA interference (RNAi), which points to their fundamental importance in coordinating plant defense, metabolism, and development. Despite the discovery of PD more than 100 years ago, and our increasing recognition of their functional significance, the underlying components and mechanisms of PD trafficking remain poorly understood. Hence, an unbiased genetic strategy is taken to dissect these molecular components and mechanisms, using an ingenious gain-of-trafficking system.

The maize KNOTTED1 (KN1) homeodomain protein was the first plant protein found to selectively traffic through PD, and its trafficking appears to be important for its function in shoot stem cell maintenance. A gain-of-function trafficking assay in *Arabidopsis* was developed to demonstrate that the carboxy-terminal region of KN1 (“KN1C”), which contains the homeodomain, is necessary and sufficient for KN1 trafficking in vivo. The trafficking assay relies on complementation of leaf hair (trichome) development in hairless *glabra1* (*gl1*) mutants. GL1, a MYB transcription factor, is required in the epidermis for trichome initiation and acts cell autonomously. However, expression of a fusion between GL1 and KN1C in cells underlying the epidermis can rescue trichomes in a *gl1* mutant background because the fusion protein can traffic through PDs into the epidermal cells. Thus, this system provides a simple and tractable model to understand how proteins traffic cell to cell. Factors critical for KN1 trafficking can be uncovered through isolating mutants defective in trichome rescue.

As a proof of concept for our genetic strategy, combining mapping and illumina-based sequencing, one mutant with attenuated KN1 trafficking (Fig. 1) was identified as a chaperonin gene. The chaperonin appears to be essential for PD trafficking of certain but not all noncell-autonomous proteins, suggesting that different mechanisms may be involved for various trafficking proteins. In addition, biochemical evidence suggests a physical association between chaperonin and KN1.

The central question now is how mechanistically a chaperonin protein may be involved in KN1 trafficking. Chaperonins have been characterized in fungal and mammalian systems as a key component of the cellular chaperone machinery, facilitating efficient protein folding in the cytosol. Proteins are thought to undergo partial unfolding during PD translocation, which makes the discovery of this chaperonin particularly exciting. Even more exciting, the hetero-oligomeric chaperonins form a cylindrical complex with a central cavity for confining the polypeptide chain and ensuring its proper folding. The chaperonin central cavity and PD microchannels have similar diameters; thus, one very exciting possibility is that chaperonins or chaperonin-like proteins may belong to the long sought-after core constituents of

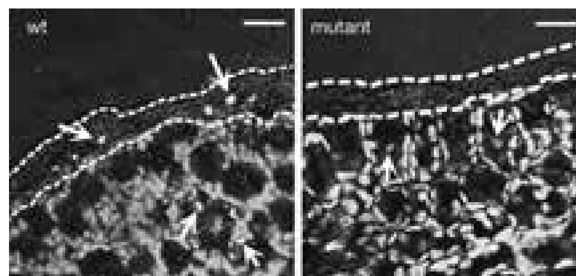

FIGURE 1 A mutant in cell-to-cell transport of KNOTTED1. Impaired PD trafficking of KN1 is demonstrated by reduced GFP~GL1~KN1C fluorescence in the chaperonin mutant leaf epidermis (outlined with dashed lines) compared to wild type (wt, arrowed). Note normal fluorescence levels in mesophyll cell nuclei in both samples (arrowed). Confocal images of hand-sectioned leaf samples are shown. Bars, 20 µm.

PDs. In support of this idea, chaperonins have been observed to associate with membranes. To address this possibility, fluorescent-protein-tagging assays are in progress to investigate the precise subcellular localization of chaperonins in *Arabidopsis*. Meanwhile, it is also possible that chaperonins may function during post-translocation protein folding and/or in maintaining proteins in an unfolded state that is competent for PD transport. The latter scenario would be analogous to posttranslational endoplasmic reticulum (ER) translocation. Various complementation experiments are under way that aim to distinguish between these possibilities.

In summary, a functional characterization of chaperonins—the first-ever factor so far known to be critical for KN1 PD trafficking—will further our understanding of developmental regulation and mechanisms of selective cell-to-cell trafficking. In addition, it may give mechanistic insights into this elaborate protein-folding machinery, which is not well understood in any system at a molecular level but has been linked to several pathological states in humans, such as cancer, sensory neuropathy, and Huntington's disease.

Hormone Signaling in Maize

S. DeBlasio, T. Zadrozny [in collaboration with A. Chan, The J. Craig Venter Institute, and A. Sylvester, University of Wyoming]

Similar to animal systems, hormones have a key role in the proper growth and development of plants. The formation and patterning of lateral organs (i.e., roots, leaves, and flowers) in crop species such as maize depend on hormonally induced changes in gene transcription, which occur within meristematic stem cells. How well these organs develop under normal and stressful environmental conditions often has major effects on important agronomic processes including crop yield and nutritional content.

To better understand the role hormone signaling has in maize development, we have created stable transgenic lines, natively expressing fluorescent-tagged versions of proteins involved in three important hormone pathways: auxin, cytokinin, and jasmonic acid. We use these lines to determine the tissue specificity and subcellular localization of the fusion proteins, documenting changes in their expression patterns throughout development and in response to various exogenous signals. Protein localization is also assessed in developmentally defective genetic backgrounds, and where possible, these fluorescent protein fusion lines have been used to complement their respective mutant alleles to verify functional activity. The lines developed thus far have provided unprecedented microscopic views of maize cellular architecture, and we can now study real-time dynamics of cell structure, function, and protein localization for ~50 tagged genes.

As an example, cytokinins act to maintain the population of stem cells within the meristem as well as promote their differentiation by interacting with plasma-membrane-bound histidine kinase receptors. This initiates a phosphorelay cascade within the cell where the kinase receptor phosphorylates a histidine phosphotransfer protein, inducing its translocation from the cytoplasm to the nucleus where it activates B-type response transcriptional regulators (RRs). This leads to changes in gene expression of proteins involved in organ differentiation, including cell elongation, chloroplast differentiation, and cell division. B-type RRs also induce transcription of A-type RRs that feed back to negatively inhibit the pathway. To date, our lab has generated fluorescent-tagged lines for at least one member of the cytokinin-specific protein families mentioned above, as well as cytokinin oxidases, which degrade the hormone, and putative cytokinin responsive proteins such as the cell cycle control protein cyclin D. Preliminary results suggest that the subcellular localization of some of these proteins may differ between organ types and is dependent on hormone levels. For example, ZmRR7-YFP (an A-type RR) is localized mainly in the cytoplasm and around the nucleus of root cells. However, in leaf epidermal cells, a greater concentration of ZmRR7-YFP was observed within the nucleus when exogenous cytokinin was applied. During tassel development, ZmRR7-YFP localizes to a discrete zone in the center of the spikelet meristem, suggesting its involvement in stem cell maintenance. We are currently working to create a cytokinin reporter line that will allow us to detect changes in cytokinin levels in planta.

We are also starting to explore the role jasmonic acid (JA) may have in maize inflorescence development. Although JA is better known for being a key signaling molecule involved in plant defense responses, recent evidence has shown that it is also involved in sex determination. Mutations in 13-lipoxygenase (LOX), one of the enzymes involved in the biosynthesis of volatile compounds, including JA, lead to the feminization of the male tassel. Application of exogenous JA to developing mutant tassels was sufficient to rescue stamen growth, suggesting that JA is required for proper male floral development in maize. Interestingly, our studies have found that the natively expressed YFP (yellow fluorescent protein) fusion protein of another member of the

13-lipoxygenase family, ZmLOX10, localizes to a discrete domain subtending spikelet pair meristems in the tassel. This pattern is similar to the one that we observed for RAMOSA3, a protein involved in inflorescence branching. At a later stage in tassel development, ZmLOX10-YFP can be observed at the boundary between spikelet meristem pairs. In collaboration with Dr. Michael Kolomiets at Texas A&M University, we are in the process of determining the function of ZmLOX10 and/or JA in early inflorescence development.

We have also generated a series of marker lines that highlight different subcellular compartments, including vacuole, nuclei, peroxisomes, microtubules, plastids, and various other structures that are available to the scientific community for study. We plan to use these markers to analyze changes that occur within the cell during differentiation and development. In some cases, the expression of fluorescent marker proteins in generated lines is so robust that it can be observed with the naked eye. For example, corn kernels natively expressing the endosperm-specific-gene α-*zein* fused to RFP (red fluorescent protein) have a distinct pink color due to the high levels of the tagged protein that have accumulated within the kernel, making identification of transgenic seeds as well as quantification of fusion protein levels easy. We are currently using this line to rapidly assess the effect that certain epigenetic mutations have on transgene silencing.

Regulation of Phyllotaxy in Maize

R. Johnston, E. Jimenez

Different plant species each has a characteristic ordered arrangement of leaves along the stem. The arrangement of leaves on the shoot axis is termed phyllotaxy. These patterns can be traced back to events in the shoot apical meristem (SAM), the reservoir of stem cells at the shoot tip that is the site of leaf initiation. The aim of our research is to investigate the mechanisms that determine the pattern of leaf initiation, specifically, interactions between developmental genes and hormone signaling pathways. The maize plant exhibits a very ordered pattern of growth, making it an excellent model for the study of phyllotaxy. Leaves of the main axis are initiated singly in an alternate pattern, generating a distichous phyllotaxy. *aberrant phyllotaxy 1* (*abph1*) mutants have an altered phyllotaxy; leaves are initiated in pairs in a decussate pattern, rather than the normal distichous pattern. This has been attributed to the enlarged SAMs of *abph1* mutants. Previous work by our lab has shown that *abph1* encodes a cytokinin-inducible type-A response regulator that is involved in the negative regulation of cytokinin signaling, indicating a role for cytokinin in phyllotactic patterning.

The plant hormone auxin is also implicated in the regulation of leaf initiation and phyllotaxy. Polar auxin transport is facilitated by the PIN1 family of proteins. Recent data indicate that PIN1 and auxin levels are reduced in *abph1* embryos. To elucidate interactions between auxin and ABPH1, we are using transgenic lines expressing the maize PIN1 protein fused to a YFP (ZmPIN1a-YFP) and ABPH1 fused to an RFP (ABPH1-RFP). Confocal microscopy was used to observe the localization of ZmPIN1a-YFP during early development of normal and *abph1* embryos. We found that in normal embryos, ZmPIN1a-YFP is expressed at high levels in the central and lower regions of the SAM before initiation of the first leaf. ABPH1-RFP is expressed in a partially overlapping domain in the central and upper part of the SAM.

Surprisingly, in *abph1* embryos, ZmPIN1a-YFP expression in the SAM was considerably delayed and reduced. Similarly, ZmPIN1a-YFP expression was not seen in the SAM before initiation of the first leaf in these mutants. We also found that initiation of the first leaf was delayed by approximately 1 day in *abph1* embryos. ZmPIN1a-YFP was expressed at similar levels and in a similar pattern in other regions of *abph1* embryos, indicating that alterations in ZmPIN1a-YFP expression were specific to the stem cell population and confirming that the *abph1* embryos that we observed were expressing the ZmPIN1a-YFP construct. Taken together, these observations support a model in which reduced auxin levels and altered PIN1 localization in the shoot meristems of *abph1* mutants delay leaf initiation, contributing to the enlarged stem cell pool and altered phyllotaxy of these mutants. We previously found that cytokinin induces *ZmPIN1a* expression in the maize SAM. To determine whether this induction occurs throughout the SAM or is confined to cells that will form the next leaf, we quantified ZmPIN1a-YFP fluorescence intensity in plants treated with cytokinin. We found that ZmPIN1a-YFP induction occurs specifically in the group of cells that will give rise to the next leaf. This result provides additional evidence for cross talk between auxin and cytokinin signaling in the regulation of phyllotaxy.

To further understand the regulation of maize phyllotaxy, we are characterizing a second mutant, *Abph2*, which is dominant. However, unlike *abph1*, *Abph2* phyllotaxy is initially normal and changes to decussate at

about leaf 5. The change to decussate phyllotaxy is often preceded by the production of a fused leaf. *Abph2* embryonic SAMs are considerably larger than normal, suggesting that like *abph1*, *Abph2* regulates phyllotaxy in part through regulation of the stem cell pool.

The identity of *Abph2* is not known and efforts to identify the gene by positional cloning are ongoing. *Abph2* was mapped to a region between two genetic markers on chromosome 7. Because there is a gap in the sequence available for this region of the maize genome, we used sequence similarity (synteny) with the rice genome and additional genetic markers to further map *Abph2* to an interval spanning two bacterial artificial chromosomes (BACs). The location of *Abph2* on these two BACs has been narrowed to an interval of ~180 kb containing five predicted genes, and we are currently sequencing them from the mutant to identify the *Abph2* gene.

In parallel with the map-based approach, we performed a mutagenesis screen to identify new alleles of *Abph2*. In this screen, pollen from homozygous *Abph2* plants was treated with a mutagenic compound, ethylmethanesulfate (EMS), and used to pollinate nonmutant plants. Seeds from these crosses were planted and screened as seedlings. Most such plants have the dominant *Abph2* phenotype. However, in cases where mutation has disrupted the *Abph2* allele, the progeny should appear normal. Eight such "knockout" plants were identified. Work is currently under way to sequence alleles of candidate genes in these "knockout" lines to identify mutations that may have caused the loss of *Abph2* function. Identification of such mutations will provide further clues to the identity of *Abph2*. In addition, this approach may generate an *abph2* loss-of-function mutant that will provide information about the normal role of this gene in plant development.

Investigation of Molecular Mechanisms Controlling Meristem Determinacy and Inflorescence Branching in Maize

S. DeBlasio, A. Goldshmidt, M. Pautler

We are interested in studying the development of maize inflorescence because its architecture is critical to reproductive success. This has a wide range of implications in hybrid seed production and ultimately crop yield. The shape of maize inflorescence is controlled by the fate and activity of axillary meristems, which subtend the suppressed leaves of the tassel and ear. The classical *ramosa* (*ra1, 2, 3*) mutants have highly branched inflorescences, due to increased activity of spikelet pair meristems. *RA1* and *RA2* encode transcription factors, but *RA3* is predicted to be a trehalose sugar metabolic enzyme. Although RA3 is a functional trehalose-6-phosphate phosphatase (TPP) in vitro and in vivo in yeast, trehalose levels are unchanged in the *ra3* mutant. This, together with several other lines of evidence, suggests that RA3 could have an additional unsuspected function in maize. First, *RA3* has a discrete expression pattern subtending axillary meristems, suggesting a developmental, rather than general, metabolic role. Second, *RA3* is genetically upstream of *RA1* and regulates its expression. Finally, RA3 immunolocalization shows that the protein is both nuclear and cytoplasmically localized and forms a speckled pattern. A yeast two-hybrid screen was conducted in order to gain a better understanding of how RA3 functions. Approximately 3.1 million clones were screened from an inflorescence cDNA library. These results of this screen suggest that RA3 interacts with several transcription factors, including those belonging to the zinc finger homeodomain (ZF-HD) class. We therefore believe that RA3, such as some metabolic enzymes in yeast (e.g., hexokinase), may have an additional role in transcriptional regulation.

To test this hypothesis, we plan to confirm RA3 interacting proteins in planta with immunoprecipitation experiments using epitope-tagged transgenic lines. Further characterization of the putative interactors, including expression analysis via in situ hybridization and reverse genetics, is currently in progress. We will also directly assay whether RA3 occupies the promoter of *RA1* in vivo via chromatin immunoprecipitation (ChIP) and explore whether nuclear localization of RA3 is required for normal development.

As an independent genetic approach, we are mapping a naturally occurring genetic suppressor of *ra3*, as well as screening EMS-mutagenized populations for additional modifiers. This approach should place additional factors in the *ramosa* pathway and provide functional insight. We also hope to exploit standing natural variation in maize inbreds to screen for further modifiers of *ra3*.

An eventual goal is to understand the mechanisms controlling determinacy of the spikelet pair meristems. Previous studies showed that both auxin transport and control of stem cell maintenance might affect meristem determinacy. We will assess behavior of the auxin transport and activity fluorescent markers in wild-type and *ramosa* mutants. In parallel, we will observe expression patterns of the maize meristem maintenance genes in all of the mutants and attempt to establish a genetic link between *ramosa* mutants and auxin or meristem maintenance genes by double-mutant analysis. As a complementary strategy, we are using a genomics approach by

comparing Illumina-based transcriptome profiles of developing wild-type and mutant ears. Our analysis will facilitate better understanding of the mechanisms controlling maize meristem determinacy and potentially define additional genes participating in the process.

Resolving Regulatory and Metabolic Networks in Meristem Determinacy during Maize Inflorescence Development

A.L. Eveland, N. Satoh-Nagasawa, D. Ware [in collaboration with Mary Beatty and Hajime Sakai, DuPont Crop Genetics]

In maize, male and female flowers develop separately in branched clusters called inflorescences as tassels and ears, respectively. Inflorescence architecture is determined by the developmental fate of axillary meristems that are composed of highly organized stem cell populations. In both immature ears and tassels, a series of axillary meristems are initiated sequentially along a developmental gradient. These include spikelet pair meristems (SPM), spikelet meristems (SM), and tassel-specific branch meristems (BM). Disruptions in SM and SPM determinacy, as shown with a core set of mutants, result in altered branching patterns. Genetic control of branching, especially in the ear where kernels are borne, has clear relevance to crop improvement programs with respect to seed number and harvesting ability. The purpose of this work is to use existing data sets and bioinformatics to computationally resolve regulatory and metabolic networks that modulate developmental fate of maize inflorescence meristems.

RAMOSA (*RA*) genes specifically affect meristem determinacy in the SPM. As mentioned in the previous section, *RA1* and *RA2* encode transcription factors and *RA3* encodes a trehalose-6-phosphatase. The latter suggests a putative link between developmental and metabolic processes. Although genetic studies in these mutants have identified key genes that regulate inflorescence architecture and their basic molecular mechanisms, links to associated metabolic and regulatory pathways have not been resolved on a genome-wide scale. In this study, a framework for gene expression is constructed based on information from a series of existing data sets. These data sets include experimental analyses of gene expression during young ear and tassel development, as well as comparisons of transcript profiles among relevant mutants. Expression data were collected by microarray analyses and sequence-based transcript profiling using Illumina's deep sequencing platform.

We hypothesized that a sequence-based approach to expression profiling would enhance specificity by distinguishing between closely related transcripts and identifying new genes expressed in developing inflorescences. To test this, we used Illumina's digital gene expression (DGE) to compare genome-wide expression profiles in B73 (normal) and *ra3* mutant maize. The DGE tag-profiling strategy targets the 3′ ends of expressed sequences to generate millions of 20-base sequence reads. Read frequency of a given sequence tag associated with a particular transcript can be used to quantify mRNA levels. Deep sequencing of immature ear samples yielded ~27 million reads with frequencies spanning four orders of magnitude. Two separate restriction digests (DpnII and NlaIII) were used to construct the DGE libraries and enabled resolution of 18,154 and 25,777 unique transcripts, respectively. Approximately 80% of these unique sequences mapped perfectly to the maize reference genome, whereas ~6% mapped to repetitive regions. Coordinates for these mappings were used to associate the unique sequences with gene models and annotations in maize. Our analyses identified differentially expressed genes and resolved expression profiles for low-expressed genes, including both *RA1* and *RA3*.

Current results from this study demonstrated the effectiveness of DGE-based transcript profiling in a highly duplicated genome such as maize and resolved differential expression profiles that will help to define the roles of the *RA3* gene. Further analyses include comparisons of *ra1* and *ra2* mutants as well as sequential stages of young ear and tassel development using DGE. In addition, another Illumina-based method, RNA-seq, will be used to sequence the entire transcriptome of immature ear and tassel samples and will provide a scaffold to anchor the short DGE tags, additional support for annotation of expressed genes, and enhanced coverage of transcript variants.

Computational methods are being used to analyze the global expression information provided by the assembled microarray- and Illumina-based data sets. These analyses include clustering methods to identify coexpressed gene sets during normal developmental progression and in response to a specific mutant background (condition dependent). On the basis of these analyses, de novo coexpression networks will be modeled. Existing genomic information, such as Gene Ontology annotations, will be integrated to assess functional commonalities among coregulated groups of genes called modules. Comparative analyses will be used to make functional predictions based on conservation of these coregulated gene sets with known signal-

ing and biochemical pathways available for rice and *Arabidopsis*. Existing information from these comparable biological systems will be leveraged to unknown modules in maize.

The resulting expression framework will provide a resource that can be continuously referenced and updated as additional data sets become available. In addition, this framework will further be integrated with genome-wide sequence analyses, chromatin-binding information, and protein–protein interaction data from the *RA* genes and other key transcriptional regulators involved in inflorescence architecture. Resulting models will provide testable hypotheses for resolving key biological questions at the interface between development and metabolism.

A Link between *fasciated ear2* Activity, Seed Row Number, and Maize Domestication

P. Bommert, Y.K. Lee, S. Choi

In this project, we follow a possible connection between the activity of the *fasciated ear2* gene and the remarkable morphological changes observed along the domestication of inflorescences in maize. In teosinte, the ancestral form of maize, kernels are arranged in two alternating rows, whereas modern elite maize hybrid lines have up to 24 rows of seeds. Kernel row number is dependent on the number of meristems, called spikelet pair meristems (SPMs), initiated by the inflorescence meristem. Our previous analysis of the *fasciated ear2* (*fea2)* gene showed that it encodes a receptor protein and that it is involved in the regulation of the inflorescence meristem, because ears of *fea2* mutants develop more than 40 rows of seed (Fig. 2).

It is tempting to speculate that the number of SPMs is directly dependent on the size of the inflorescence meristem. As expected, we did find a significant positive correlation between inflorescence meristem size and kernel row number variation based on the analysis of 15 randomly chosen maize inbred lines. This indicates that the control of inflorescence meristem size has a strong impact on kernel row number, which is, as mentioned above, one of the major morphological changes between teosinte and maize and one of the key traits in modern breeding programs. To address whether allelic variation in the *fea2* gene also has an impact on seed row number via the control of meristem size, we performed a high-resolution quantitative trait locus (QTL) analysis, using 250 intermated maize recombinant inbred lines, each grown in three repetitions in three consecutive years. The analysis supports the hypothesis that allelic variation in *fea2* regulates seed row number, because the most significant QTL is located on maize chromosome 4, corresponding to the *fea2* map position. We are currently analyzing *fea2* expression levels within the inflorescence meristem in the parental lines of the QTL population and expect to find a correlation between *fea2* expression level and inflorescence meristem size.

Besides this quantitative genetics approach, we were also looking for hypomorphic or weak *fea2* alleles, to further substantiate our hypothesis. Using the maize TILLING facility we were able to isolate four new EMS-induced *fea2* alleles, which are currently being evaluated for a possible impact on seed row number. We expect to find weak *fea2* alleles with an increase in seed row number, but without the abnormal fasciated phenotype observed in null alleles.

We are also pursuing the isolation of other fasciated genes in maize, such as *fasciated ear3 (fea3*) and *compact plant2 (ct2)*. Using ~900 *ct2* F_2 mutants, we have

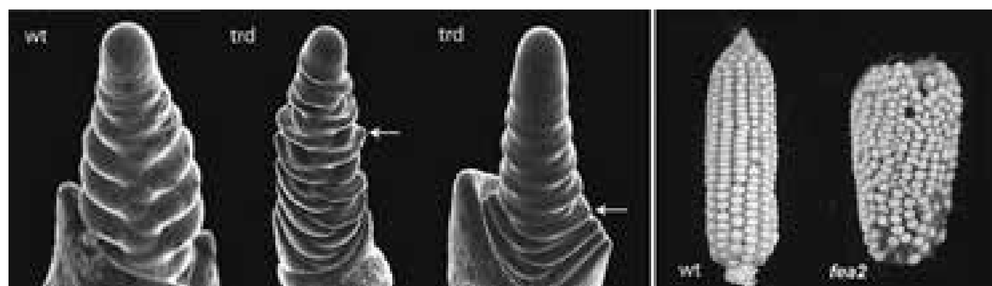

FIGURE 2 Mutants affecting inflorescence architecture and seed row number. (*Left panel*) Scanning electron micrographs of a wild-type and two *third outer glume* (*trd*) mutants of barley. *trd* mutants show ectopic growth of leaves in the inflorescence (arrowed), and we have found that this gene encodes an ortholog of the maize *tasselsheath1* gene, also identified in our lab. (*Right panel*) Mature ears of wild-type and the *fasciated ear2* mutant (*fea2*). Note that *fea2* ears have an increased number of vertical seed rows on the ear.

narrowed down the chromosomal location of this gene to an interval of ~250 kb on chromosome 1, which is highly syntenic to rice and sorghum. This region is covered by two BAC clones containing eight predicted genes. Subsequent sequencing of these genes in the *ct2-Muszynski* allele led to the identification of a transposon insertion in a *lipoxygenase*-encoding gene. However, none of the other *ct2* alleles showed a lesion in this gene. We therefore isolated additional *ct2* alleles via a targeted EMS screen. By screening a total of 33,000 individuals, we were able to isolate three new *ct2* alleles. Sequencing of these alleles is in progress.

grassy tillers1 and *tasselsheath1*: Two Genes Controlling Maize Plant Architecture

C. Whipple, K. Chen, J. Yuan

When plants make the transition from vegetative to reproductive growth, they undergo dramatic morphological changes. One such change that is seen in many plant species, including the model plants maize and *Arabidopsis*, is the suppression of leaf growth in the inflorescence. These inflorescence leaves are known as bracts, and suppression of bract growth has evolved multiple times in diverse plant lineages. We have been investigating the genetic mechanism controlling bract suppression in maize to better understand how leaf development is controlled in plants and to understand how novel developmental pathways evolve.

We have identified several mutants representing five distinct genes that, when mutated, fail to suppress bract growth in maize. Two of these genes, *tasselsheath1* and *tasselsheath4*, have been cloned, and we are currently investigating how they act together to control the maize bract suppression pathway. Our results indicate that *tasselsheath4* is expressed first and is necessary for *tasselsheath1* expression. Mutants similar to *tasselsheath1* are known in rice and barley (Fig. 2), and we have shown that in both cases, the gene corresponding to *tasselsheath1* gene is mutated. This indicates that bract suppression by *tasselsheath1* is a conserved mechanism throughout the grass family. Interestingly, the genes corresponding to *tasselsheath1* and *tasselsheath4* in *Arabidopsis* have no role in bract suppression. Because the grasses evolved from a common ancestor that had bracts, we are investigating the possibility that the *tasselsheath* pathway evolved early in the evolution of grasses. How developmental pathways arise to generate morphological novelties is a fundamental biological question that has received little attention, and the *tasselsheath* mutants of maize represent a powerful model to investigate this. Additionally, the emerging pathway for bract suppression in the grasses is distinct from that described in *Arabidopsis*. Because bract suppression evolved independently in these lineages leading to the grasses and *Arabidopsis*, our results suggest that there are multiple ways to repress bract growth in plants.

In addition to our work on bract suppression, we have been investigating a maize mutant with increased branch number and branch length. We have shown that this mutant is identical to the classical maize mutant *grassy tillers1*, named for its profuse production of basal branches or tillers. The *grassy tillers1* gene is expressed in young branch buds and is necessary to induce a dormant state that is responsive to light cues. Plants are unable to move, and thus, they must adjust their developmental pathways in response to environmental signals. For example, plants in the shade tend to suppress branching and elongate in order to reach open light. Plants sense shade by measuring the amount of far-red wavelength light that they receive. Interestingly, *grassy tillers1* is induced by far-red light, suggesting that it is normally turned on by shade to suppress branching. Consequently, *grassy tillers1* represents an interesting link between plant environmental responses and an important agronomic developmental pathway.

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PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen	A.-Y. Chang	D. Irvine	R. Schwab	M. Tanurdžić
	P. Finigan	A. Kloc	R. Shen	M.W. Vaughn
	E. Gruntman	S. Locke	J. Simorowski	J. Xue
	J.-J. Han	U. Ramu	R.K. Slotkin	M. Zaratiegui
	K. Hansen	M. Regulski		

Heterochromatic silencing, gene control, and stem cell function in plants and fission yeast provide useful models for epigenetic inheritance, differentiation, and disease in higher organisms. In fission yeast, RNA interference (RNAi) of centromeric transcripts regulates histone modification, whereas in *Arabidopsis*, methylation of transposons and repeats depends on small RNA and chromatin remodeling. In yeast, these mechanisms function during chromosomal replication and have a role in epigenetic inheritance. In *Arabidopsis,* mobile *trans*-acting small interfering RNAs (siRNAs) arise from transposons in cultured cells and in pollen, and they regulate cell fate via noncoding RNA in the leaf. In addition to our work on epigenetics and development, we continue to develop transposons and artificial microRNA (miRNA) as tools to probe genomic function in plants. During the past year, we were joined by graduate students Patrick Finigan and Joe Calarco, and said goodbye to Sarahjane Locke and Eyal Gruntman. Postdoc Klavs Hansen joined the lab from the University of Copenhagen, and Rebecca Schwab left for Strasbourg at the end of the year. Mike Regulski joined the lab as part of the Plant Group genomics collaboration.

RNAi Guides Histone Modification during the S Phase of Chromosomal Replication

A. Kloc, M. Zaratiegui

For many decades after its initial characterization, heterochromatin was considered to be transcriptionally inert, but we have found that this highly condensed chromosomal material is transcribed, and rapidly silenced, by an orchestrated sequence of events directed by RNAi during the S phase of the cell cycle. Together, these findings suggest a model of RNAi-directed epigenetic inheritance. Heterochromatin remains condensed throughout the cell division cycle and silences genes nearby, but the mechanism by which heterochromatin is inherited has remained obscure. Heterochromatic silencing and histone H3 lysine-9 methylation (H3K9me2) depend, paradoxically, on heterochromatic transcription and RNAi. We have shown that heterochromatin protein 1 in fission yeast (Swi6) is lost via phosphorylation of H3 ser-10 (H3S10) during mitosis, allowing heterochromatic transcripts to transiently accumulate in S phase. Rapid processing of these transcripts into siRNA promotes restoration of H3K9me2 and Swi6 after replication when cohesin is recruited. RNAi in fission yeast is inhibited at high temperatures, providing a plausible mechanism for epigenetic phenomena that depend on replication and temperature, such as vernalization in plants and position-effect variegation in animals. These results explain how "silent" heterochromatin can be transcribed and lead to a model for epigenetic inheritance during replication.

Mapping Mutations in Fission Yeast Using Whole-genome Next-generation Sequencing

D. Irvine, M.W. Vaughn [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory; Derek Goto, Hokkaido University; and Mitsuhiro Yanagida, Kyoto University]

Fission yeast is an important model for cell cycle and epigenetic studies due to the ease with which genetic mutants can be isolated. However, forward genetic mutant screens are impaired by difficulties complementing mutants with genomic libraries, whereas reverse genetic screens generally exclude essential genes. Epigenetic phenotypes are typically unstable, preventing complementation if silencing cannot be reestablished. We have shown that resequencing the fission yeast genome following mutagenesis can readily identify mutations generated by forward genetic approaches. Candidate genes were identified as functional single-nucleotide polymorphisms (SNPs) linked to the mutation and then used to recapitulate the mutant phenotype. Next-generation genomic resequencing promises to dramatically enhance the power of yeast genetics, permit-

ting the isolation of subtle alleles of essential genes and alleles with quantitative effects, as well as enhancers and suppressors of heterochromatic silencing.

Heterochromatin, Histone Modification, and RNAi in Fission Yeast

M. Zaratiegui, D. Irvine, K. Hansen, S. Locke, A.-Y. Chang, M.W. Vaughn [in collaboration with Yang Shi, Harvard Medical School, and W. Zac Cande, University of California, Berkeley]

We are applying a combination of forward and reverse genetics, genomics, and biochemistry to further explore the link between RNAi, histone modification, and heterochromatic silencing in the fission yeast *Schizosaccharomyces pombe*. In most eukaryotes, histone methylation patterns regulate chromatin architecture and function: Methylation of histone H3K9 demarcates heterochromatin, whereas H3K4 methylation demarcates euchromatin. In one example, the JmjC-domain protein Lid2 is a trimethyl H3K4 demethylase responsible for H3K4 hypomethylation in heterochromatin. Lid2 interacts with the histone lysine-9 methyltransferase Clr4 through the Dos1/Clr8-Rik1 complex, which also functions in the RNAi pathway. Disruption of the JmjC domain alone results in severe heterochromatin defects and depletion of siRNA, whereas overexpressing Lid2 enhances heterochromatin silencing. The physical and functional link between H3K4 demethylation and H3K9 methylation suggests that the two reactions act in a coordinated manner. Surprisingly, cross-regulation of H3K4 and H3K9 methylation in euchromatin also requires Lid2. Lid2 enzymatic activity is thus regulated through a dynamic interplay with other histone-modification enzymes. Our findings provide mechanistic insight into the coordination of H3K4 and H3K9 methylation.

Epigenetic Reprogramming and Small RNA Silencing of Transposable Elements in Pollen

K. Slotkin, M. Tanurdzic, M. Vaughn [in collaboration with J. Becker and J. Feijo, Gulbenkian Institute, Portugal]

Heterochromatin is composed of transposable elements (TEs) and related repeats. Like TEs, heterochromatin silences genes located nearby and has a major role in epigenetic regulation of the genome. The mutagenic activity of TEs is suppressed by epigenetic silencing and siRNAs, especially in gametes that could transmit transposed elements to the next generation. In *Arabidopsis* pollen, we have found that TEs are unexpectedly reactivated and transpose but only in the vegetative nucleus, which accompanies the sperm cells but does not provide DNA to the fertilized zygote. TE expression coincides with down-regulation of the heterochromatin remodeler *DECREASE IN DNA METHYLATION 1* and of many TE siRNAs. However, 21-nucleotide siRNAs from *ATHILA* retrotransposons are generated in pollen and accumulate in sperm, suggesting that siRNA from TEs activated in the vegetative nucleus can target silencing in gametes. We propose a conserved role for reprogramming in germ-line companion cells, such as nurse cells in insects and vegetative nuclei in plants, to reveal intact TEs in the genome and regulate their activity in gametes.

Epigenomic Consequences of Immortalized Cell Suspension Culture and Polyploidy in Arabidopsis

M. Tanurdžić, R.K. Slotkin, P. Finigan, E. Gruntman, M. Vaughn [in collaboration with W. Thompson, North Carolina State University; L. Comai, University of California, Davis; R.W. Doerge, Purdue Unviersity; and V. Colot, Ecole Normale Superieure, Paris, France]

Plant cells grown in culture exhibit genetic and epigenetic instability. In a continuously proliferating, dedifferentiated cell suspension culture of *Arabidopsis,* we have found that euchromatin becomes hypermethylated and that a small percentage of these genes become associated with heterochromatic marks. In contrast, the heterochromatin undergoes dramatic and very precise DNA hypomethylation with transcriptional activation of specific TEs. High-throughput sequencing of siRNA revealed that TEs activated in culture have increased levels of 21-nucleotide siRNA, resembling pollen in this respect (Fig. 1). In contrast, TEs that remain silent retain the predominant 24-nucleotide siRNA class and do not change significantly in their siRNA profiles. Increases in euchromatic DNA methylation and 21-nucleotide siRNA are accompanied by changes in gene expression: loss of the DNA demethylase *REPRESSOR OF SILENCING1* (*ROS1)* and gain of *ARGONAUTE2*, respectively; but the loss of TE DNA methylation is selective and levels of *DDM1* remain high. These results implicate RNAi and chromatin modification in epigenetic restructuring of the genome following the activation of TEs in immortalized cell culture. Polyploidy in plants also results in genetic and epigenetic changes in gene expression from generation to generation. The

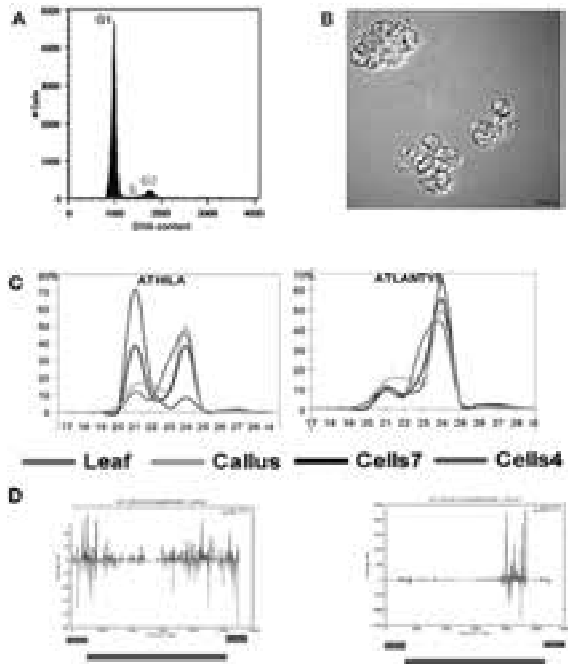

FIGURE 1 Small RNA profiles of immortalized cell cultures of *Arabidopsis*. Plant cells grown in suspension culture are diploid by fluorescence-activated cell sorting (FACS) analysis (*A*) and are transferred to fresh media every 7 d (*B*) and harvested after 16 h (Cells7) or 4 d (Cells4). (*C*) Small RNA from *ATHILA* retrotransposons are predominantly 21 nucleotides in cell culture, but 24 nucleotides in leaf and callus; 24-nucleotide small RNA from *ATLANTYS* retrotransposons are unaffected in cell culture. (*D*) Small RNA from *ATHILA* elements in leaves (*left*) and cell culture (*right*) were matched to Watson (*above*) and Crick (*below*) strands and are 10–100 times more abundant in cell culture. A schematic of the *ATHILA* retrotransposon with LTRs (long terminal repeats) is indicated below.

mechanisms that underlie these changes are important in the short term (hybrid sterility) as well as in the long term (hybrid vigor), because polyploids are stabilized during evolution. We are using microarrays and next-generation Illumina sequencing to profile chromatin modifications and small RNA in synthetic allopolyploids from crosses between tetraploid *A. thaliana* and tetraploid *A. arenosa*.

Artificial miRNAs

R. Schwab [in collaboration with D. Weigel, Tübingen, and W.R. McCombie and G. Hannon, Cold Spring Harbor Laboratory]

RNAi is a powerful tool for gene discovery, and the latest RNAi vectors contain short hairpins that are modified precursors of miRNAs. These produce a distinct small RNA sequence, which can be modified and optimized, and is often called artificial miRNA (amiRNA). A systematic approach is currently being used to generate thousands of hairpins to silence the majority of transcripts encoded by *Arabidopsis*. Numerous successful applications of amiRNAs have been reported, but ~25% of these amiRNAs did not trigger gene silencing. We are investigating the nature of unsuccessful gene silencing in *Arabidopsis* by directly analyzing the effect of local secondary structures in target transcripts and also by an unbiased search for additional determinants.

Mobile *Trans*-acting siRNA in *Arabidopsis*

R. Schwab [in collaboration with M. Byrne, John Innes Center; M. Crespi, Orsay; and O. Voinnet, Strasbourg]

Plants encode an additional class of small RNAs, called *trans*-acting siRNAs (tasiRNAs), that posttranscriptionally regulate protein-coding genes, as do miRNAs. tasiRNA biogenesis requires multiple components of the siRNA pathway and also miRNAs. We previously isolated a gene-trap reporter insertion in *TAS3a*, which is limited to the adaxial (upper) side but targets *AUXIN RESPONSE FACTOR (ARF)* genes throughout the leaf (CSHL Annual Report 2006). We have now shown by in situ hybridization and reporter gene fusions that the silencing activities of ARF-regulating tasiRNAs control ARF activities noncell autonomously in the shoot and in the root. In situ hybridization in mutant backgrounds indicates that the mobile signal is likely to be either the tasiRNA itself or an unstable double-stranded RNA intermediate. One component of this regulatory network is the SANT-domain gene *ASYMMETRIC LEAVES1 (AS1)*. *as1* mutants are dramatically enhanced by mutants in the tasiRNA pathway (CSHL Annual Report 2006). We have found three additional genes, *PIGGYBACK1 (PGY1), PGY2,* and *PGY3*, that alter leaf patterning in the absence of *AS1*. All three *pgy* mutants develop dramatic ectopic lamina outgrowths on the adaxial side of the leaf in an *as1* mutant background. This leaf-patterning defect is enhanced by mutations in the adaxial homeodomain gene *REVOLUTA (REV)* and is suppressed by mutations in abaxial *KANADI* transcription factor genes. *PGY1, PGY2,* and *PGY3* encode cytoplasmic large-subunit ribosomal proteins L10a, L9, and L5 and are expressed on the adaxial side of the leaf, suggesting a role for translation.

Maize-targeted Mutagenesis: A Knockout Resource in Maize

J.-J. Han, M. Regulski, M. Vaughn [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory, and P. Ferreira, University of Rio de Janeiro]

The maize-targeted mutagenesis (MTM) population comprises seed and tissue from 44,000 maize plants in which *Mutator* transposons have been mobilized and then stabilized genetically. Tissue was harvested in 18 two-dimensional "grids" of 48 x 48 plants each, and genomic DNA was prepared from row and column pools that permit cross-referencing of any individual sample. Target sites flanking each *Mutator* insertion can be amplified en masse from these pools by a ligation-mediated polymerase chain reaction (PCR). Sequencing using next-generation Illumina GA2 paired-end reads, followed by mapping back to the maize genome, resulted in identification of a high proportion of the flanking sites, including control insertions previously recovered by conventional means. By first comparing nonintersecting row pools, and then row and column pools, it is possible to distinguish preexisting (parental) insertions from new germinal insertions at row-column intersections. We estimate that ~0.5 to 1 million independent new insertions of *Mutator* elements in the gene-rich portion of the genome can be identified with only a few hundred sequencing reactions.

Gene Trapping and Chromatin Charting in *Arabidopsis*

J. Simorowski, U. Ramu, R. Shen, M. Vaughn [in collaboration with D. Spector and W.R. McCombie, 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 40,000 individual insertions, more than half of which have been mapped to the genome by PCR and sequencing. The resulting knockouts, along with phenotypic and expression data, are made available to the public via TRAPPER (http://genetrap.cshl.edu/), an interactive database and ordering system. Reporter genes are silenced by position-effect variegation when they are located a few kilobases from the NOR (nucleolar organizer) and depend on histone deacetylation, but not chromatin remodeling, mimicking nucleolar dominance in this respect. A very sharp boundary (< 100 bp) separates silent and active insertions near NOR4. We have expanded this system to include gene-trap transposons with *lacR*-GFP (green fluorescent protein) chromosome-charting beacons that enable chromosomal locations to be visualized in the nucleus. These can be used to visually track individual loci in living plants while simultaneously producing a genome-wide "transcription potential map" via a common luciferase expression cassette. A direct correlation between mobility constraint, subnuclear location, and gene activity was observed in some cases. We provide a collection of almost 300 *Arabidopsis* lines for examining epigenetic control in living cells within chromatin regions at the resolution of a few kilobases (http://charting.cshl.edu). Our studies revealed regional gene silencing near a heterochromatin island via DNA methylation that is correlated with mobility constraint and nucleolar association. We also found an example of nucleolar association that does not correlate with gene suppression, suggesting that distinct mechanisms exist that can mediate interactions between chromatin and the nucleolus. These studies demonstrate the utility of this resource in unifying structural and functional studies toward a more comprehensive model of how global chromatin organization coordinates gene expression.

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Jong-Jin Han

PLANT DEVELOPMENTAL GENETICS

M. Timmermans	D. Chitwood	C. Hu	E. Plavskin
	M. Dotto	A. Husbands	A. Sarkar
	F. Dupuy	M. Lodha	D. Skopelitis
	C. Fernandez-Marco	F. Nogueira	

Plants have the distinctive ability to form new organs throughout their lifetime, which can span hundreds or even thousands of years. The growing tip of a plant contains a population of stem cells that are located within a specialized niche, termed the shoot apical meristem (SAM). These stem cells divide to maintain the SAM and to generate daughter cells from which lateral organs, such as leaves and flowers, arise. The research in our lab aims to dissect the genetic networks that operate within the SAM to regulate stem cell homeostasis and to generate and pattern leaves.

Adaxial-Abaxial Patterning by ASYMMETRIC LEAVES1 (AS1) and AS2

A. Husbands, F. Nogueira

Outgrowth and patterning of lateral organs in plants depend on the specification of adaxial-abaxial (upper/lower) polarity. We have shown that this asymmetry is generated by a novel patterning mechanism in which small RNAs establish opposing fates of the axis. In maize, microRNA (miRNA) miR390 accumulates on the adaxial (upper) side of developing leaves where it triggers the biogenesis of *TAS3*-derived *trans*-acting short interfering RNAs (ta-siRNAs). These confine the accumulation of abaxial determinants, including AUXIN RESPONSE FACTOR3 (ARF3) and miR166, to the lower side of leaves. miR166, in turn, demarcates the abaxial side by repressing expression of class III homeodomain leucine zipper (HD-ZIPIII) transcription factors that specify adaxial fate.

Our findings indicate that the establishment of organ polarity requires the precise spatiotemporal accumulation and relative efficacy of *TAS3*-derived ta-siRNAs and miR166. We are using genetic and biochemical approaches to identify genes that regulate the accumulation and activities of these polarizing small RNAs. In *Arabidopsis*, several lines of evidence indicate that the DNA-binding proteins AS1 and AS2 form a complex that contributes to organ polarity. We have used chromatin immunoprecipitation (ChIP) to identify polarity determinants that are direct AS1-AS2 targets, and these include selected *MIR166* precursors and components of the *TAS3* ta-siRNA pathway. Our ChIP results have been verified using reporter constructs and reverse transcriptase–polymerase chain reaction (RT-PCR) analysis.

The abaxial determinants *ARF3* and *ARF4* are targets for tasiR-ARFs, a subset of *TAS3*-derived ta-siRNAs. We have shown that transcript levels for both *ARF* genes are up-regulated in *as2*. Considering that *TAS3* ta-siRNA pathway components are direct targets of AS1-AS2, these transcription factors may control *ARF3* and *ARF4* expression through regulation of tasiR-ARF accumulation. However, mutants defective in both the tasiR-ARF and AS1/AS2 pathways show an enhanced leaf phenotype, suggesting that both pathways converge to repress *ARF3* and *ARF4* activity on the adaxial leaf surface. To test the interaction between the AS1/AS2 and tasiR-ARF pathways, we generated *as2* plants expressing a tasiR-ARF–insensitive allele of *ARF3* (*ARF3m*). Double mutants develop highly serrated leaves with abaxialized ectopic leaf-like outgrowths at the margins. Double mutants defective for *AS2* and ta-siRNA biogenesis components (e.g., *as2 rdr6* or *as2 sgs3*) also acquire this phenotype, but they are significantly delayed in doing so. Our hypothesis is that the hyperaccumulation of *ARF3*, in the presence of a defective *AS* pathway, results in the production of ectopic leaflets on margins. RT-PCR experiments confirm that *ARF3* levels are dramatically higher in *as2 ARF3m* double mutants than in *as2 rdr6* or *as2 sgs3* double mutants, which may explain why ectopic leaflets form much earlier in this genotype. We are currently examining the putative connection among the tasiR-ARF pathway, auxin transport, and the production of compound leaf architecture.

The START Domain Regulates Class III HD-ZIP Activity and Organ Polarity

A. Husbands

The HD-ZIPIII transcription factors—PHABULOSA (PHB), PHAVOLUTA (PHV), and REVOLUTA (REV)—

are required and sufficient for the specification of adaxial cell fate. Their role as adaxial determinants was uncovered through dominant mutations that prevent the cleavage of *PHB, PHV,* and *REV* transcripts by miR166. Consequently, mutant transcripts accumulate on both sides of developing leaf primordia, resulting in their adaxialization. HD-ZIPIII proteins contain a putative START lipid/sterol-binding domain, suggesting that the function of HD-ZIPIII proteins may be regulated by an unknown ligand.

Several *pPHB:PHB-YFP* reporter constructs were created to address whether the START domain is indeed important for proper PHB function. Because recessive loss-of-function *phb* mutants display no distinguishing phenotypes, *pPHB:PHB-YFP* reporters also carry silent point mutations in the miR166-binding site. We then assayed whether substitution of two highly conserved arginine residues in the START domain or deletion of the START domain entirely suppresses the leaf phenotypes. Point mutations in the miR166-binding site alone conditioned a strong adaxialized leaf phenotype in most T1 plants, as expected. Mutations in the critical arginine residues reduced the frequency of this extreme phenotype, whereas deletion of the START domain mostly resulted in formation of normal leaves. These data suggest that the START domain is critical for PHB function. We are investigating potential mechanisms through which the START domain may regulate PHB activity, such as (1) subcellular localization, (2) DNA-binding affinity, and (3) protein stability. In addition, we will use a modified yeast–one-hybrid approach to screen a small-molecule library for putative ligands of PHB and other HD-ZIPIII proteins.

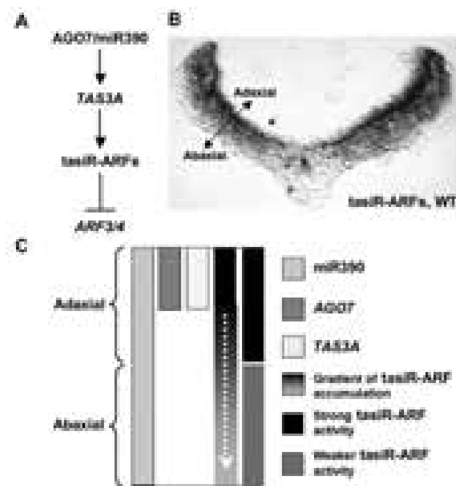

FIGURE 1 Pattern formation in leaves via small RNA mobility. (*A*) Diagram of the tasiR-ARF pathway. (*B*) In situ hybridization using an LNA (locked nucleic acid) probe reveals a gradient of tasiR-ARF accumulation that is strongest on the adaxial (upper) side of the leaf and dissipates away toward the abaxial (lower) side. (*C*) Summary of the localization patterns of tasiR-ARF pathway components. Although miR390 accumulates uniformly throughout leaves, tasiR-ARF biogenesis is restricted to the few adaxial-most cell layers of leaves by the localized expression of *AGO7* and *TAS3A*. tasiR-ARFs accumulate outside this defined domain of biogenesis and form a gradient across leaves (*B*), indicative of mobility of this small RNA (dotted arrow). The tasiR-ARF gradient is interpreted into domains of strong and weak activities along the adaxial-abaxial axis, leading to the polarized expression of the abaxial determinant ARF3.

Pattern Formation via Small RNA Mobility

D. Chitwood, F. Nogueira [in collaboration with T. Montgomery, Dr. M. Howell, and Dr. J. Carrington, Oregon State University, Corvallis]

A recurring theme in pattern formation is the interpretation of relative distance from a point source through the activity of mobile, positional signals. siRNAs that are produced upon transgene-induced gene silencing or during viral infection are known to move systemically throughout plants, but intercellular movement of developmentally relevant endogenous small RNAs has not been observed. By comparing the pattern of tasiR-ARF accumulation with the localization of tasiR-ARF biogenesis components (Fig. 1), we sought to determine whether this unique class of endogenous siRNAs is mobile and can traffic between cells from their source of biogenesis. Using an ARF3-based sensor of tasiR-ARF activity, we showed that tasiR-ARFs act throughout the leaf, but more strongly on the upper (adaxial) side than on the lower (abaxial) side. tasiR-ARFs thereby delimit expression of the abaxial determinant ARF3 discretely to the lower side of the leaf. In situ hybridization analysis revealed that miR390, whose activity initiates tasiR-ARF biogenesis, accumulates ubiquitously in leaves. In contrast, the tasiR-ARF biogenesis factor *ARGONAUTE7* (*AGO7*) and the tasiR-ARF precursor *TAS3A* both localize exclusively to the two adaxial-most cell layers. Consequently, tasiR-ARF biogenesis is restricted to the adaxial side of developing leaves where miR390 colocalizes with AGO7 and *TAS3A*. However, tasiR-ARF activity is detected outside this region of biogenesis, indicating that tasiR-ARFs traffic from the adaxial side to the abaxial side. Indeed, in situ hybridization analysis revealed that tasiR-ARFs accumulate in a gradient across leaves that is strongest adaxially and dissipates toward the abaxial side of the leaf. This small

RNA gradient is interpreted into adaxial and abaxial domains of high and low tasiR-ARF activity that creates a discrete pattern of ARF3 expression on the abaxial side of the leaf (Fig. 1).

These data identify the first mobile positional signal in adaxial-abaxial patterning of the leaf. Moreover, our study reveals two novel RNA interference (RNAi)-based patterning mechanisms. First, subspecialized interactions between RNAi effector proteins and small RNAs, such as that between miR390 and AGO7, can act to spatially limit small RNA activity during development. Second, the movement of small RNAs, such as tasiR-ARFs, outside of their domain of biogenesis, precisely patterns targets and might serve to convey positional information, demonstrating that small RNAs can serve as mobile, inductive signals during development.

Dissecting Small RNA Mobility in Plants

D. Chitwood, D. Skopelitis

An intriguing question is whether miRNAs, like endogenous siRNAs, can traffic between cells. Given the scope of miRNA-regulated gene networks in development, the cell-to-cell movement of miRNAs would have important implications with respect to their potential to act as inductive signals and generators of pattern. Using in situ hybridization and *pMIR390:GUS* reporter constructs, we have shown that expression of both *MIR390* precursors is limited to developing leaf primordia and the region below the SAM, whereas mature miR390 accumulation extends into the meristem proper. Although expression of artificial miRNAs from a vascular-specific promoter suggests phloem-derived miRNAs act cell autonomously in mature leaves, the shoot apex may provide a unique developmental context that permits the intercellular movement of miRNAs, thus providing a possible explanation for the disparity between the accumulation patterns of miR390 and its precursors in the meristem. At least one other miRNA, miR391, shows a similar discrepancy between the localization pattern of the mature miRNA and its precursor in the meristem region. Unlike miR390, miR391 possesses a 5′ U and is loaded into AGO1, suggesting that the possible movement of miR390 would not be an outcome of its unique recruitment into an AGO7 complex, but a general property of miRNAs expressed beneath the SAM.

We are taking advantage of miRNA sensors to further investigate the possible movement of miRNAs in a variety of developmental contexts. Similar experiments are also under way to further investigate ta-siRNA mobility. Such experiments will reveal parameters of small RNA mobility, such as tissue specificity and direction and range of movement, and whether it is dose dependent. We anticipate that small RNAs move from cell to cell through intercellular channels known as plasmodesmata and that unique proteins mediate mobility. We have designed a forward genetic screen to identify such potential small RNA mobility factors and hope to report on these in next year's report.

The Ancestral Role of the ta-siRNA Pathway

E. Plavskin

Genes involved in ta-siRNA biogenesis, as well as miR390 and the *TAS3* loci themselves, are conserved in the moss *Physcomitrella patens*. Even more interesting, ta-siRNAs produced from *Physcomitrella TAS3* loci target transcripts of *AUXIN RESPONSE FACTOR* genes. Basal land plants such as *Physcomitrella* lack true leaves, and their "leaf-like" lateral outgrowths are composed of a single cell layer. The ancestral role of the *TAS3* ta-siRNA pathway will thus be distinct from its known role in adaxial-abaxial patterning of leaves in higher plants. To dissect the developmental processes in moss regulated by tasiR-ARFs, we are characterizing knockouts of genes involved in ta-siRNA biogenesis in *Physcomitrella*, as well as the targets of the moss tasiR-ARFs. This will reveal the ancestral role of this unique small RNA pathway.

Novel ta-siRNA Loci in Maize

M. Dotto

In plants, miRNAs and ta-siRNAs form through distinct biogenesis pathways. ta-siRNAs are derived from *TAS* precursor transcripts that are cleaved by miRNA-guided ARGONAUTE proteins, either AGO1 or AGO7. *Arabidopsis* contains 10 members of the ARGONAUTE family and four characterized *TAS* gene families (*TAS1* to *TAS4*). AGO1 is required for the biogenesis of ta-siRNAs from *TAS1* and *TAS2* loci, whereas AGO7 is uniquely required for the biogenesis of *TAS3* ta-siRNAs. We previously identified four *TAS3* loci in the maize genome. Thus far, these are the only known maize genes giving rise to ta-siRNAs. Our interest is to investigate whether additional *TAS* loci exist in maize. We found

that the maize genome encodes 20 AGO proteins, with only one homolog of AGO7 and four AGO1 homologs. We are generating transgenic plants expressing FLAG-tagged versions of AGO7 or AGO1 to characterize the RNAs that interact with these proteins. We expect these to include ta-siRNAs, miRNAs that trigger ta-siRNA production, other miRNAs, and possibly larger transcripts that are targets of small RNA regulation. As a comparison, and for additional information regarding the small RNA pathways in maize, we will also analyze one of the two maize AGO10 homologs. This ARGONAUTE protein has been implicated in the maintenance of the SAM and in the modulation of leaf development in *Arabidopsis.*

Global Expression Analysis of Meristem Function and Leaf Initiation

A. Sarkar [in collaboration with M. Scanlon, Cornell University, Ithaca, New York; P. Schnable, Iowa State University, Ames; B. Buckner and D. Janick-Buckner, Truman State University, Kirksvile, Missouri; and D. Ware, Cold Spring Harbor Laboratory]

leafbladeless1 (*lbl1*) encodes an essential component in the ta-siRNA biogenesis pathway in maize. Mutations in *lbl1* lead to formation of radial, abaxialized leaves. To gain insight into gene networks regulated by ta-siRNAs and/or involved in adaxial-abaxial patterning, we used laser capture microdissection coupled with microarray analyses to compare the global gene expression profiles in the SAM and first leaf of wild-type and *lbl1* mutants. We identified 352 genes whose expression levels are significantly altered in *lbl1*, including (1) components of the ta-siRNA pathway, which suggests that potential feedback regulation exists in this pathway; (2) known determinants of adaxial-abaxial polarity, which is consistent with the abaxialization of *lbl1* leaves; (3) known developmental regulators; and (4) novel maize genes.

Surgical experiments and our small RNA expression studies revealed a critical contribution by the epidermal (L1) layer to adaxial-abaxial patterning. Interestingly, 25 genes that are differentially expressed between wild type and *lbl1* are also differentially expressed between the L1 and subepidermal (L2) meristem layers. These include genes predicted to function in auxin or lipid signaling. In situ mRNA localization data confirm that *arf3a*, a direct ta-siRNA target, localizes preferentially to the epidermis and abaxial side of wild-type leaf primordia and is more broadly expressed in *lbl1*. Another predicted auxin-regulated gene as well as a lipid-transfer protein are, likewise, L1 enriched in wild-type and ectopically expressed in *lbl1* mutant apices. A role for such signaling pathways in organ polarity has often been postulated, but insights into these developmental mechanisms are lacking.

lbl1 acts in the incipient leaf (P0) to specify adaxial identity. We identified 47 genes that are differentially expressed between wild-type and *lbl1* meristems as well as between the incipient leaf and the SAM proper. Adaxial domain-enriched expression patterns of predicted developmental regulators, such as *yabby15* and *seven-absentia-like,* are changed in *lbl1* mutants. Expression of a predicted developmental regulator, *squamosa promoter binding protein-like,* is enriched in the abaxial domain of normal leaf primordia but accumulates ectopically in *lbl1* mutants, consistent with a possible role in adaxial-abaxial patterning. This is currently being tested by expression analysis in other maize leaf polarity mutants. We are also taking a bioinformatics approach to identify potential novel ta-siRNA targets among those genes up-regulated in *lbl1* apices. This project will provide novel insights into the gene networks controlled by ta-siRNAs and/or involved in leaf polarity.

Establishment of Determinacy during Organ Development

M. Lohda, C. Hu

Indeterminacy of the SAM is maintained in part by the class I *KNOX* homeobox genes. To give rise to determinate structures, such as leaves, *KNOX* gene expression must be maintained in a stable “off” state throughout lateral organ development. We have shown earlier that this process requires a complex that includes the transcription factors AS1 and AS2 as well as the chromatin-remodeling factor HIRA. Using ChIP experiments, we previously identified two sites in the promoters of the *Arabidopsis KNOX* genes *BP* and *KNAT2* that bind the AS1-AS2-HIRA repressor complex. Complex binding is mediated via the *cis*-regulatory motifs CWGTTD-KMKTTGAHW. Promoter deletion analysis revealed that both complex-binding sites are essential and act together to maintain the “off” state of the *BP* promoter. In addition, these promoter deletion studies identified a putative leaf enhancer located between the AS1-AS2-HIRA complex-binding sites in the *BP* promoter. Indeed, when placed upstream of a minimal promoter, this predicted enhancer region is capable of driving reporter gene expression in leaves. On the basis of these results, we proposed that the AS1-AS2-HIRA complex

creates a loop around the leaf enhancer and recruits additional chromatin-remodeling factors to repress enhancer activity throughout organogenesis.

We have now identified a role for the CLF-MSI1-FIE-EMF2 type of polycomb repressive complex 2 (PRC2) in the stable silencing of *KNOX* genes during leaf development. Consistent with the notion that PRC2 complexes have histone H3K27 trimethylation activity, we have identified this repressive mark on nucleosomes at the *BP* and *KNAT2* promoters in leaves of *Arabidopsis,* where these genes are silent. Besides, in *clf* mutant plants, levels of this H3K27 trimethylation mark are reduced and *KNOX* genes are misexpressed in developing leaves. In animal models, PRC1 is recruited to sites of H3K27 trimethylation established by PRC2. In *Arabidopsis, LIKE HETEROCHROMATIN PROTEIN 1 (LHP1)* is suggested to have a role similar to that of PRC1. Consistent with this view, we have identified *BP* and *KNAT2* as direct targets of LHP1 and have shown that *KNOX* genes are misexpressed in *lhp1* mutants. We are currently investigating whether the AS1-AS2-HIRA complex directs the recruitment of the PRC2 complex to the *KNOX* loci. These data will provide insight into the epigenetic mechanism that maintains stem cell homeostasis in plants. Because HIRA and the PRC2 complexes are also implicated in the regulation of the stem cell state in mammals, our findings in plants may provide a useful framework for future studies on HIRA- and PcG-mediated gene silencing in mammalian embryonic stem cells and induced pluripotent stem cells.

Regulation of Embryonic Patterning by HIRA

C. Fernandez-Marco, F. Dupuy [visiting Master's student from the Université Paris Diderot, Paris, France]

Arabidopsis plants with reduced *HIRA* transcript levels develop leaves that resemble the leaves of *as1* and *as2* mutants and that ectopically express several *KNOX* genes. However, null alleles of *HIRA* lead to early embryo lethality, suggesting that *HIRA* regulates expression of target genes that function in developmental processes other then the *KNOX* genes. To further dissect the role of *HIRA* in plant development, we have isolated 12 independent *hira* alleles carrying substitutions in highly conserved amino acids. These mutant alleles exhibit a range of developmental phenotypes, including defects in embryo, root, and vegetative development. Thus far, much of our phenotypic analysis has focused on *hira3,* which contains a point mutation in the evolutionary conserved fifth WD40-repeat motif. The endosperm is affected in *hira3* mutants, showing enlarged and irregular nuclei. In addition, *hira3* mutants are marked by abnormal patterns of cell division in the hypophysis. This highly specialized cell at the boundary between embryo and extraembryonic suspensor normally divides to form a lens-shaped cell that ultimately gives rise to the quiescent center (QC) of the root stem cell niche. The phytohormone auxin has a key role in the establishment of apical-basal embryo polarity, specification of the hypophysis, and the subsequent development of the root stem cell niche.

To determine the molecular basis for the embryonic root defects, we are taking advantage of molecular markers to examine the effects of *hira3* on cell-fate specification and patterning of the early embryo. Preliminary data show that expression of the auxin efflux carrier PIN7 is reduced and shifted basally, such that, in *hira3,* it is lost from the hypophysis and upper cells of the suspensor. Similarly, expression of the synthetic DR5 reporter, which marks auxin maxima, no longer coincides with the position of the presumptive hypophesis but is displaced to basal cells of the suspensor. Finally, expression of the auxin responsive gene *IAA11*, which normally marks the hypophysis, is absent in *hira3* mutants. Together, these data suggest that auxin transport is altered in *hira3* such that an auxin maxima is no longer established at the boundary between the proembryo and suspensor and that this perturbs specification of the hypophysal cell.

Later in root development, the QC is positioned by the overlap between two independent inputs: PLETHORA (PLT) expression, which is activated in response to the local auxin maxima, and SCARECROW/SHORTROOT, which have a role in radial patterning of the root. One of the genes specifically expressed in the QC is the homeobox gene *WOX5*. In situ hybridization experiments revealed that expression of these genes is also reduced or lost in *hira3*. The QC signals to adjacent stem cells in the root meristem to prevent their differentiation. Although *hira3* mutant embryos are not normally viable, mutants can be rescued when fertilized ovules are transferred onto culture medium. Consistent with the above-mentioned defects, rescued mutant seedlings lack a root. A similar set of defects have been reported for the root mutants *monopteros* and *bodenlos*. We are currently testing the genetic interactions between *hira3* and *mp*, and further phenotypic analyses of *hira3* are still ongoing. The outcome of these experiments will allow us to position HIRA in the genetic network that patterns the root stem cell niche during embryogenesis.

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Damianos Skopelitis

Naman Patel

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 3 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 interactions 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.

The success of the program is apparent from the list of distinguished alumni. Previous Cold Spring Harbor Laboratory Fellows Adrian Krainer (1986), Scott Lowe (1995), and Marja Timmermans (1998) are now members of the faculty at the Laboratory, and Marja is the Director of the Fellows program. After completing her fellowship, Carol Greider (1988) joined the CSHL faculty, but after 9 years, she moved to Johns Hopkins University School of Medicine where she is now the Daniel Nathans Professor and Director of Molecular Biology and Genetics. Eric Richards (1989) currently heads a research group at the Boyce Thompson Institute for Plant Research at Cornell University, and David Barford (1991) is Professor of Molecular Biology at the Institute of Cancer Research in London. Ueli Grossniklaus (1994) also was a member of our faculty before leaving to become a Professor at the Institute of Plant Biology, Universität Zürich, Switzerland. Térence Strick (2000) left at the end of his fellowship to become a Group Leader at the Institut Jacques Monod, Centre National de la Recherche Scientifique and Universités de Paris VI and VII. Both Gilbert (Lee) Henry (2000) and Ira Hall (2004) moved to Virginia upon completion of their fellowships. Lee joined a project headed by Thomas Südhof at Howard Hughes Medical Institute's (HHMI) Janelia Farm in Ashburn, and Ira is Assistant Professor in the Department of Biochemistry and Molecular Genetics at the University of Virginia in Charlottesville.

The Laboratory's current Fellow, Patrick Paddison, joined the program in 2004. He was a graduate student at the Watson School of Biological Sciences, where he worked in Greg Hannon's lab on the development of RNA interference (RNAi) libraries. As a Fellow, Patrick has been using RNAi technology to establish the genetic requirements for embryonic stem (ES) cell self-renewal and differentiation. His report can be found below.

Late this fall, two new Fellows joined the Lab: Florin Albeanu did his graduate studies at Harvard University, in the laboratories of Venki Murthy and Markus Meister, where he investigated the logic of odor maps in the olfactory bulb of rodents. As a Fellow, Florin is taking advantage of optical imaging, electrophysiology, and opto-genetic tools to understand what computations occur at the neural circuit level in the olfactory bulb of behaving mice. Chris Vakoc joined us from Gerd Blobel's laboratory at the University of Pennsylvania. As a Fellow, Chris is studying the role of histone lysine methylation in normal and malignant hematopoiesis.

Functional Genetics in Cancer and Stem Cell Systems

P.J. Paddison

There are more than 200 different cell types in the human body, each with a specialized function, which arise during development and adult tissue homeostasis from transient or established progenitor cells resident in tissues and organs. Two emerging themes in disease research emphasize why it is crucial that we understand how cell identities are formed and maintained in mammals. First is the notion that cancer cells may arise from maligned development programs. In addition to coopting growth and survival promoting pathways, tumor cells hijack molecular pathways that are normally involved in developmental processes such as cell-fate determination. The existence of cancer stem cells, which may have vital roles in tumor progression, maintenance, and recurrence, underscores this notion. Second is the

notion that with the successful isolation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), we can develop techniques to harness their developmental potential in the laboratory for clinical applications, such as cell replacement therapies for neurodegeneration, spinal chord injury, liver dysfunction, severe burns, and blood disorders.

The overall goals of the Paddison lab revolve around using functional genetics approaches to uncover the molecular networks that regulate mammalian cell identity. Through the use of defined in vitro embryonic and somatic stem cell systems, we will find and characterize gene products affecting stem cell self-renewal, differentiation, proliferation, and survival. These studies will provide insights into basic cell biology, stem cell biology, tissue homeostasis, and signaling network design and perhaps provide inroads for implementing targeted cellular therapies for a range of human diseases.

To capture the dynamic interplay between nuclear factors that modulate "epigenetic" states and contextual factors that alter basic cellular processes in our studies, we have incorporated sets of small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) targeting ~1700 predicted transcription and DNA-binding factors and ~8000 genes with predicted enzymatic activity. Outlined below are three of the projects currently under way in the lab, which seek to uncover the functional genetic properties of embryonic stem cells (ESCs), neural stem cells (NSCs), and transformed cells.

SELF-RENEWAL AND LINEAGE DETERMINATION OF ESCS

ESCs are cell lines derived from the inner cell mass (ICM) of blastocyst-stage mammalian embryos (Evans and Kaufman, *Nature 292:* 154 [1981]; Thomson et al., *Science 282:* 1145 [1998]; Ludwig et al., *Nat. Biotechnol. 24:* 185 [2006]). They can grow indefinitely in culture and give rise to cells of all three embryonic germ layers as well as germ cells (Keller, *Genes Dev. 19:* 1129 [2005]). For these reasons, ESCs hold great promise for regenerative medicine. Some molecular details of the ESC self-renewal network have emerged, but more in-depth knowledge will be required to facilitate future ESC-based clinical applications.

The homeodomain transcription factor Nanog has a central role in maintaining the pluripotency of ESCs and is exclusively expressed in pluripotent cells, i.e., ESCs or primordial germ cells (Chambers et al., *Cell 113:* 643 [2003]; Mitsui et al., *Cell 113:* 631 [2003]). To gain further insight into ESC pluripotency networks, we performed a loss-of-function RNA interference (RNAi) screen in a Nanog-GFP (green fluorescent protein) ESC reporter line. We identified several novel activators and repressors of ESC self-renewal gene expression. Chief among them were core members of the SWI/SNF complex (i.e., Smarcc1 and Smarce1). In eukaryotes, the SWI/SNF complex uses the energy from ATP hydrolysis to alter chromatin structure and transcriptional regulation by affecting interactions between chromatin and chromatin-bound nucleosomes (Vignali et al., *Mol. Cell. Biol. 20:* 1899 [2000]). SWI/SNF function has been linked to diverse gene regulatory phenomena in eukaryotes, ranging from mating type switching in yeast to neural development in mammals (Sudarsanam and Winston, *Trends Genet. 16:* 345 [2000]; Roberts and Orkin, *Nat. Rev. Cancer 4:* 133 [2004]; Lessard et al., *Neuron 55:* 201 [2007]). We have shown that SWI/SNF acts as both a corepressor of self-renewal gene expression and a coactivator of differentiation/lineage-specific genes. Thus, SWI/SNF acts as an important and general coordinator of changes in ESC fate and perhaps an integrator of several distinct modes of epigenetic regulation. To identify additional genes required for either ESC self-renewal or lineage commitment, we have expanded this screen to a genome-scale screen using two approaches. The first approach involves a well-by-well siRNA screen (9835 genes) for genes that regulate Oct4/Pou5f1 expression in mESCs under normal growth conditions. A second approach uses bar-code array analysis of pools of retroviruses bearing ~70,000 different shRNAs to identify those that bypass ESC differentiation (i.e., genes required for differentiation) (Paddison et al., *Nature 428:* 427 [2004]). The aims of these projects are to (1) find additional key regulatory factors governing ESC self-renewal and differentiation, (2) characterize the SWI/SNF-dependent epigenetic determinants of pluripotency, and (3) identify and characterize genes that are rate-limiting for hESC and iPSC derivation and self-renewal.

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WATSON SCHOOL OF BIOLOGICAL SCIENCES

ADMINISTRATION

Leemor Joshua-Tor, Ph.D., *Professor and Dean*
Uwe Hilgert, Ph.D., *Assistant Dean (until November 2008)*
Dawn Meehan, B.A., *Director, Admissions and Student Affairs*
Alyson Kass-Eisler, Ph.D., *Curriculum Director and Postdoctoral Program Officer*
Kim Geer, *Administrative Assistant*

EXECUTIVE COMMITTEE

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Leemor Joshua-Tor

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Uwe Hilgert (ex officio, until November 2008)
W. Richard McCombie
Alea A. Mills
David L. Spector (ex officio)
Nicholas Tonks
Linda Van Aelst

Student Representatives
Eyal Gruntman, WSBS (until October)
Jane Lee-Osborne, SBU (until October)
Eugene Plavskin, WSBS (from November)
Xiaoyun Wu, SBU (from November)

Secretary
Alyson Kass-Eisler

ADMISSIONS COMMITTEE

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Gregory Hannon

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Professor and Executive Vice Dean, School of Medicine
University of California, San Francisco

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Investigator, Howard Hughes Medical Institute

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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

Yet another year has flown by here at the Watson School of Biological Sciences (WSBS). It is hard to believe that we are now in our historic tenth year!

In October 2007, the School appointed Uwe Hilgert, Ph.D., as the School's Assistant Dean. Dr. Hilgert had previously been at CSHL's Dolan DNA Learning Center (DNALC). In November 2008, Uwe returned to the DNALC to participate in the exciting new iPlant initiative. We thank him for his year of dedication to the School.

In fall 2008, Alyson Kass-Eisler, WSBS Postdoctoral Program Officer, was promoted to Curriculum Director. Alyson has been with the School since 2003 and she continues to organize our postdoctoral programs and graduate student curriculum. She will also have a larger role in the School's reporting responsibilities. Dawn Meehan, Director of Admissions and Student Affairs, has also been with the School since 2003. Dawn has an essential role in the successful recruitment of outstanding students worldwide. She also brings a unique personal touch to the School to ensure that the needs of our students are met while they pursue their rigorous studies. Kimberley Geer rounds out "Team Watson" as our Administrative Assistant.

Team Watson: (*Left to right*) Alyson Kass-Eisler, Dawn Meehan, Leemor Joshua-Tor, and Kim Geer.

Faculty Changes

In 2008, we welcomed Mickey Atwal, Thomas Gingeras, Bo Li, Zachary Lippman, Pavel Osten, and Darryl Pappin to the School as new faculty members.

Thomas Gingeras, Professor and the Head of the Functional Genomics program, comes to us as a former Vice President of Biological Research at Affymetrix, Inc. A leader in functional genomics, his research focuses on understanding what really defines a gene and how genomes are organized and regulated. He has already had an impact on the teaching at the School as an instructor in the *Genetics and Genomics* Specialized Disciplines course in fall 2008.

Assistant Professor Gurinder (Mickey) Atwal came to the Watson School from the Institute for Advanced Study at Princeton University, where he was a postdoctoral fellow. Mickey is our first new hire in the exciting field of quantitative biology. His research will integrate computational, analytical, and experimental data to study the evolution and diversity of genomes.

Bo Li, an Assistant Professor, first came to CSHL as a postdoctoral fellow under the supervision of former faculty member Professor Roberto Malinow. His research will look into the question of how glutamatergic synapse dysfunction can lead to the pathogenesis of psychiatric disorders including schizophrenia and depression.

Assistant Professor Zachary Lippman is a WSBS alumnus, having received his degree from the School in 2005. Zach conducted his thesis research at CSHL under the direction of Professor Robert Martienssen. He then moved to Israel to conduct research on tomato biology under the direction of Dr. Dani Zamir at the Hebrew University. Zach's laboratory will focus on understanding flower development using the tomato as a model system. We are fortunate to have Zach participate as a member of the School's Admissions Committee beginning with the entering class of 2009.

Pavel Osten, a new Associate Professor, came to the Laboratory from Northwestern University, where he held an Assistant Professorship. The main research interest in Pavel's laboratory is the identification and analysis of brain regions, neural circuits, and connectivity pathways that are disrupted in genetic mouse models of autism and schizophrenia.

Darryl Pappin joined the Laboratory as an Associate Professor. He was until recently a Scientific Fellow at Applied Biosystems, having trained at the University of Leeds in the United Kingdom. Darryl is an

expert in proteomics, and in addition to leading his research program analyzing complex biological samples, he will be leading the Laboratory's proteomics core facility.

We are very excited to have each of these exciting newcomers work with our students—a few have already become members of student Thesis Committees and several have participated as guest lecturers in our fall courses. We look forward to their participation in additional WSBS activities in the future.

Four faculty members departed the Laboratory in 2008: Hollis Cline, Zachary Mainen, Roberto Malinow, and Vivek Mittal. As faculty members, all four served the School and its students in various roles, including instructor, guest lecturer, rotation advisor, mentor, examiner, and thesis committee member. Additionally, Holly Cline was a critical member of the School's Executive Committee for 3 years. We will miss our fellow colleagues, and we wish them well in their new endeavors.

The Fifth WSBS Graduation

Graduation is the highlight of the year, and April 13, 2008 saw the Watson School's fifth graduation ceremony. The graduating class of 2008 comprised Hiroki Asari, Rebecca Bish, François Bolduc, Monica Dus, Angélique Girard, Christopher Harvey, and Wei Wei, all from the entering class of 2003, who were awarded the Ph.D. degree. Claire Biot and Adrienne Jones, from the entering class of 2006, were awarded a Master's degree. The graduation was once again a wonderful occasion, where honorary degrees were bestowed on Nobel Laureate Dr. Eric Kandel, Microsoft cofounder Mr. Paul G. Allen, and Professor of Neurology and Psychiatry Dr. Oliver Sacks, who gave this year's commencement address.

As with each graduation, we extended a special welcome to the family members and friends of our students who attend the ceremony. Among these special guests were family members who traveled from China, France, Japan, and Italy to take part in this special event.

Graduating class of 2008: (*Left to right*) Wei Wei, Monica Dus, Hiroki Asari, Christopher Harvey, Angélique Girard, François Bolduc, Rebecca Bish, Claire Biot. (Not shown: Adrienne Jones.)

2008 WSBS DOCTORAL RECIPIENTS

Student	Thesis advisor	Academic mentor	Current Position
Hiroki Asari	Anthony Zador	Z. Josh Huang	Postdoctoral fellow with Dr. Markus Meister, Harvard University
François Bolduc	Tim Tully	Hollis Cline	Assistant Professor, University of Alberta, Canada
Rebecca Bish	Michael P. Myers	Linda Van Aelst	Postdoctoral fellow with Dr. Eric Holland, Memorial-Sloan Kettering Cancer Center
Monica Dus	Gregory Hannon	John Inglis	Postdoctoral fellow with Dr. Greg Suh, NYU School of Medicine (February 2009)
Angélique Girard	Gregory Hannon	Jan Witkowski	Considering postdoctoral opportunities in France
Christopher Harvey	Karel Svoboda	Adrian Krainer	Postdoctoral fellow with Dr. David Tank, Princeton University
Wei Wei	Roberto Malinow	Jan Witkowski	Postdoctoral fellow with Dr. Marla Feller, University of California, Berkeley

2008 THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 2002

Allison Blum, July 9, 2008

rutabega *signaling in distinct circuits supports short- versus long-term memory in* Drosophila.

Thesis Examining Committee
Chair: **Grigori Enikolopov**
Research mentor: **Josh Dubnau**
Academic mentor: **David Jackson**
Committee member: **Glenn Turner**
Committee member: **J. Peter Gergen,** *Stony Brook University*
External examiner: **J. Steven deBelle,** *University of Nevada, Las Vegas*

Shu-Ling Chiu, July 14, 2008

The role of insulin receptors in the development of neuronal structure and function.

Thesis Examining Committee
Chair: **Senthil K. Muthuswamy**
Research mentor: **Hollis Cline**
Academic mentor: **Alea A. Mills**
Committee member: **Glenn Turner**
Committee member: **Linda Van Aelst**
External examiner: **Li-Huei Tsai,** *Picower Institute, MIT*

ENTERING CLASS OF 2003

Monica Dus, March 19, 2008

Beyond classical RNAi: Toward an understanding of germ cell development and transposon control in the fruitfly D. melanogaster.

Thesis Examining Committee
Chair: **Leemor Joshua-Tor**
Research mentor: **Gregory Hannon**
Academic mentor: **John R. Inglis**
Committee member: **Robert Martienssen**
Committee member: **Adrian R. Krainer**
External examiner: **J. Peter Gergen,** *Stony Brook University*

Angélique Girard, March 17, 2008

Mammalian Piwi proteins and transposon silencing in the male germ line.

Thesis Examining Committee
Chair: **Marja Timmermans**
Research mentor: **Gregory Hannon**
Academic mentor: **Jan A. Witkowski**
Committee member: **Robert Martienssen**
Committee member: **Scott Keeny,** *Memorial Sloan-Kettering Cancer Center*
External examiner: **Douglas L. Chalker,** *Washington University, St. Louis*

Christopher Harvey, February 4, 2008

Crosstalk between nearby synapses on hippocampal dendrites.

Thesis Examining Committee
Chair: **Linda Van Aelst**
Research mentor: **Karel Svoboda**
Academic mentor: **Adrian R. Krainer**
Committee member: **Hollis Cline**
Committee member: **Roger Davis,** *University of Massachusetts Medical School*
External examiner: **Steven A. Siegelbaum,** *Columbia University*

DOCTORAL THESIS RESEARCH

Student	Academic mentor	Research mentor	Thesis research
ENTERING CLASS OF 2004			
Daniel H. Chitwood *George A. and Marjorie H. Anderson Fellow*	Alea A. Mills	Marja Timmermans	The contribution of small RNAs to positional signaling and the establishment of adaxial-abaxial polarity in leaves.
Galen A. Collins *Beckman Graduate Student*	Marja Timmermans	William Tansey	Role of ubiquitin ligases and activator destruction in transcription.
Oliver L. Fregoso *Seraph Foundation Fellow* *William Randolph Hearst Scholar*	Nicholas Tonks	Adrian R. Krainer/ Michael P. Myers	Proteomic analysis to elucidate the splicing and nonsplicing roles of SR proteins.
Keisha A. John *William Randolph Hearst Fellow and Scholar*	Josh Dubnau	Linda Van Aelst	Identification of the molecular determinants contributing to DOCK7's role in neuronal polarity.
Shraddha S. Pai *Charles A. Dana Fellow*	Anthony Zador	Carlos D. Brody	Determining the neuroanatomical loci and electrical correlates of duration discrimination in the rat.
David R. Simpson *Beckman Graduate Student*	Scott Lowe	William Tansey	Revealing insights into cancer biology with tumor-derived mutations in c-Myc.
ENTERING CLASS OF 2005			
Patrick M. Finigan *Beckman Graduate Student*	Senthil K. Muthuswamy	Robert Martienssen	Epigenetic mechanisms involved in centromere function.
Amy Y. Leung *Beckman Graduate Student*	David L. Spector	William Tansey	Role of H2B ubiquitylation in chromatin localization.
Sarahjane M. Locke *George A. and Marjorie H. Anderson Fellow*	Josh Dubnau	Robert Martienssen	Histone H3 lysine 9 methylation and RNA processing in RNAi-mediated heterochromatin formation in *Schizosaccharomyces pombe.*
Hiroshi Makino *Elisabeth Sloan Livingston Fellow*	Hollis Cline	Roberto Malinow	Optical determination of the spatial distribution of experience-dependent bidirectional synaptic plasticity.
Katherine McJunkin *Robert and Teresa Lindsay Fellow*	Terri Grodzicker	Scott Lowe	Using negative-selection RNAi screens to identify novel treatment strategies for hepatocellular carcinoma.
Frederick D. Rollins *Cashin Fellow*	Jan A. Witkowski	Gregory Hannon	An RNAi screen for modifiers of cellular response to the targeted therapeutic Erlotinib.
Oliver Tam *Bristol-Myers Squibb Fellow*	David Jackson	Gregory Hannon	The role of RNAi machinery in oocyte maturation and embryonic development of the mouse.
Jeremy E. Wilusz *Beckman Graduate Student*	John R. Inglis	David L. Spector	Identification and functional characterization of large nuclear retained noncoding RNAs misregulated in breast cancer.
ENTERING CLASS OF 2006			
Yaniv Erlich *Goldberg-Lindsay Fellow*	John R. Inglis	Gregory Hannon	microRNA target identification by systematic sensor expression.
Eyal Gruntman *Elisabeth Sloan Livingston Fellow*	Josh Dubnau	Glenn Turner	Olfactory perception in *Drosophila.*
Colin Malone *Beckman Graduate Student* *NSF Graduate Research Fellow*	David J. Stewart	Gregory Hannon	Dissecting small RNA pathways in *Drosophila.*

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
Amy Rappaport *Barbara McClintock Fellow*	William Tansey	Scott Lowe	Identification and characterization of tumor suppressor genes in acute myeloid leukemia.
Claudio Scuoppo *Engelhorn Scholar*	Scott Lowe	Gregory Hannon	Identification of novel oncosuppressors through an in vivo RNAi screen in the eμ-myc model.
ENTERING CLASS OF 2007			
Megan Bodnar *Starr Centennial Scholar* **Proposal Defense: January 2009**	Nicholas Tonks	David L. Spector	The nuclear choreography of chromatin dynamics, gene expression, and gene repression in embryonic stem cells.
Ralph Burgess *Starr Centennial Scholar* **Proposal Defense: January 2009**	Bruce Stillman	Gregory Hannon	Recombination hot spots: Characterizing fine-scale variation in the frequency of meiotic recombination across the mammalian genome.
Joseph Calarco *David H. Koch Fellow* **Proposal Defense: January 2009**	David Jackson	Leemor Joshua-Tor/ Robert Martienssen	Mechanistic insights into chromatin regulating proteins in *Schizosaccharomyces pombe* and *Arabidopsis thaliana*.
Saya Ebbesen *Starr Centennial Scholarship* **Proposal Defense: January 2009**	David J. Stewart	Scott Lowe	In vivo identification and characterization of novel tumor suppressors relevant to human breast cancer.
Paloma Guzzardo *Leslie C. Quick, Jr. Fellow* **Proposal Defense: January 2009**	Adrian R. Krainer	Gregory Hannon	A potential role for small RNAs in heterochromatin formation in *Drosophila melanogaster* somatic cells.
Kyle Honegger *Crick-Clay Fellow* **Proposal Defense: January 2009**	John R. Inglis	Glenn Turner	Neuronal and circuit mechanisms creating sparse odor representations in the mushroom body of *Drosophila*.
Marek Kudla *George A. and Marjorie H. Anderson Fellow* **Proposal Defense: January 2009**	David Jackson	Gregory Hannon	3′UTR role in posttranscriptional regulation of gene expression.
Michael Pautler *William R. Miller Fellow* **Proposal Defense: February 2009**	Robert Lucito	David Jackson	The RAMOSA pathway and inflorescence branching in maize.
Maria Pineda *William Randolph Hearst Scholar* **Proposal Defense: January 2009**	Adrian R. Krainer	Raffaella Sordella	Mechanism of “addiction” to receptor tyrosine kinases in non-small-cell lung carcinoma.
Yevgeniy Plavskin *Alfred Hershey Fellow* **Proposal Defense: January 2009**	Jan A. Witkowski	Marja Timmermans	The evolution of the miR390-dependent tasiRNA pathway and its function in the development of basal land plants.
Joshua Sanders *Edward and Martha Gerry Fellow* **Proposal Defense: January 2009**	Bruce Stillman	Adam Kepecs	*Trans*-regional coordination of activity in the mouse brain.
Zhenxun Wang *A*STAR Fellow* **Proposal Defense: March 2009**	Terri Grodzicker	Adrian R. Krainer	Alternative splicing of pyruvate kinase M in tumorigenesis.
Petr Znamenskiy *David and Fanny Luke Fellow* **Proposal Defense: January 2009**	Terri Grodzicker	Anthony Zador	Pathways for attention and action in the auditory system.

Teaching Award

At this year's graduation ceremony, Dr. Glenn Turner was presented with the third annual Winship Herr Faculty Teaching Award named in honor of the School's founding Dean. Glenn was lead instructor of the neuroscience module of the *Scientific Reasoning and Logic* course and was chosen by the students for his enthusiasm, excellence, and creativity in teaching. The following is some of what they had to say about the recipient:

Glenn Turner (*front*)

> *I feel that Glenn really went out of his way to make sure that this module was enjoyable for all—from meeting with us before the module to determine what gaps needed to be filled in, to helping us with questions during our student paper discussion, to meeting with students before the final exam to review practice questions.*

Alumni Highlights

The scientific research achievements of WSBS students continue to be outstanding. As a consequence, WSBS graduates are highly competitive and are moving into faculty positions much faster, on average, than are other new Ph.D.s. WSBS graduates continue to publish in top journals and secure prestigious independent positions, fellowships, and awards. The number of WSBS graduates who either have started, or will soon start, tenure-track positions has now grown to six. Here is where are they now:

ALUMNI

François Bolduc, Ph.D.
Assistant Professor, University of Alberta, Canada

François Bolduc conducted his thesis research in Dr. Tim Tully's laboratory. His thesis was entitled "Role of fragile-X mental retardation protein and the RNAi pathway in *Drosophila* learning and memory."

Ira Hall, Ph.D.
Assistant Professor, University of Virginia School of Medicine

Ira Hall conducted his research in Dr. Shiv Grewal's laboratory. His thesis was entitled "A role for RNA interfence in heterochromatic silencing and chromosome dynamics." Ira's research contributed to the prestigious AAAS Newcomb Cleveland Prize during his time at the Watson School.

Zachary Lippman, Ph.D.
Assistant Professor, Cold Spring Harbor Laboratory

Zachary Lippman conducted his thesis research in Dr. Rob Martienssen's laboratory. His thesis was entitled "Transposons, heterochromatin, and epigenetic landscapes in *Arabidopsis thaliana*." Zach was the recipient of the prestigious Fred Hutchinson Cancer Research Center's Harold M. Weintraub Award. Zach was also the recipient of the Human Frontier Science Program Fellowship. He conducted his postdoctoral research with Dr. Dani Zamir at the Hebrew University of Jerusalem on quantitative genetics and natural variation in tomatoes.

Patrick Paddison, Ph.D.
Assistant Professor, Fred Hutchinson Cancer Center

Patrick Paddison conducted his thesis research in Dr. Gregory Hannon's laboratory. His thesis was entitled "RNA interference in mammals: The quest for illuminating gene function." During his time at the Watson School, Patrick was the recipient of a U.S. Army Medical Research and Material Comment Breast Cancer Research Program Predoctoral Fellowship.

Ji-Joon Song, Ph.D.
Assistant Professor, Korea Advanced Institute of Science and Technology

Ji-Joon Song conducted his thesis research in Dr. Leemor Joshua-Tor's laboratory. His thesis was entitled "Structural and biochemical studies of Argonaute reveal the slicing mechanism of RISC-RNAi effector complex." Ji-Joon conducted postdoctoral research with Dr. Robert E. Kingston on chromatin modification at Massachusetts General Hospital, Harvard University. Ji-Joon was the recipient of the Jane Coffin Childs Postdoctoral Fellowship.

Niraj Tolia, Ph.D.
Assistant Professor, Washington University School of Medicine, St. Louis

Niraj Tolia conducted his thesis research in Dr. Leemor Joshua-Tor's laboratory. His thesis was entitled "Structural and biochemical studies of the malaria parasite *Plasmodium falciparum* protein EBA-175 involved in erythrocyte invasion."

Admissions 2008

The School received 270 applications for the 2008–2009 academic year and is deeply indebted to its Admissions Committee, which reviewed, interviewed, and selected candidates for our doctoral program. Joining me on the Admissions Committee for the 2008 entering class were Gregory Hannon (Chair), Josh Dubnau, Adam Kepecs, Adrian R. Krainer, Robert Lucito, Nicholas Tonks, Lloyd Trotman, Linda Van Aelst, and Michael Q. Zhang—a truly remarkable team!

Entering Class of 2008

On August 26, 2008, the Watson School opened its doors for the 10th time to welcome yet another new class. This year, 15 students joined the School: Philippe Batut, Mitchell Bekritsky, Dario Bressan, Carrie Clendaniel, Melanie Eckersley-Maslin, Jiahao Huang, Sang-Geol Koh, Katie Liberatore, Ozlem Mert, Elizabeth (Beth) Nakasone, Zinaida (Zina) Perova, Felix Schlesinger, Nilgun Tasdemir, Elvin Wagenblast, and Susann Weissmueller. Reflecting CSHL's eclectic mix of nationalities, the entering class of 2008 is also international, hailing from the United States, France, Italy, Australia, South Korea, Turkey, Russia, and Germany. Beth, an American, comes from almost as far away as our foreign students—she is our first student from Hawaii.

The 2008 entering class celebrates the holidays.

ENTERING CLASS OF 2008

Philippe Batut, Université Paul Sabatier, Toulouse, France
Academic mentor: Alexander Gann

Mitchell Bekritsky, Northwestern University
Academic mentor: W. Richard McCombie

Dario Bressan, University of Pisa, Italy
Academic mentor: Gregory Hannon

Carrie Clendaniel, Northwestern University
Academic mentor: David Jackson

Melanie Eckersley-Maslin, University of Sydney
Academic mentor: David L. Spector

Jiahao Huang, University of California, Berkeley
Academic mentor: Terri Grodzicker

Sang-Geol Koh, Seoul National University
Academic mentor: Glenn Turner

Katie Liberatore, University of Mexico
Academic mentor: Adrian R. Krainer

Ozlem Mert, Bilkent University
Academic mentor: John R. Inglis

Elizabeth Nakasone, University of Southern California
Academic mentor: Alea A. Mills

Zinaida Perova, St. Petersburg State Polytechnic University
Academic mentor: Linda Van Aelst

Felix Schlesinger, Jacobs University, Bremen
Academic mentor: Thomas Gingeras

Nilgun Tasdemir, Bilkent University
Academic mentor: Josh Dubnau

Elvin Wagenblast, University of Heidelberg
Academic mentor: Jan A. Witkowski

Susann Weissmueller, University of Heidelberg
Academic mentor: Raffaella Sordella

Entering class of 2008: (*Back, left to right*) Jiahao Huang, Beth Nakasone, Melanie Eckersley-Maslin, Katie Liberatore. (*Middle, left to right*) Susann Weissmueller, Elvin Wagenblast, Felix Schlesinger, Dario Bressan, Philippe Batut, Sang-Geol Koh. (*Front, left to right*) Carrie Clendaniel, Nilgun Tasdemir, Ozlem Mert, Zina Perova, Mitchell Bekritsky.

Academic Mentoring

The Watson School takes great pride in the level of mentoring that it offers its students. One of the unique features of our program is our two-tiered mentoring approach, whereby each student receives an academic as well as a research mentor. Entering students select a member of the research or nonresearch faculty to serve as their academic mentor—a watchful guardian to look over and encourage the student through their sometimes-trying 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 our success. This year's new academic mentors for the entering class of 2008 are listed below.

STUDENT	MENTOR	STUDENT	MENTOR
Philippe Batut	Alexander Gann	Ozlem Mert	John R. Inglis
Mitchell Bekritsky	W. Richard McCombie	Elizabeth Nakasone	Alea A. Mills
Dario Bressan	Gregory Hannon	Zinaida Perova	Linda Van Aelst
Carrie Clendaniel	David Jackson	Felix Schlesinger	Thomas Gingeras
Melanie Eckersley-Maslin	David L. Spector	Nilgun Tasdemir	Josh Dubnau
Jiahao Huang	Terri Grodzicker	Elvin Wagenblast	Jan A. Witkowski
Sang-Geol Koh	Glenn Turner	Susann Weissmueller	Raffaella Sordella
Katie Liberatore	Adrian R. Krainer		

The Fall Term Curriculum

Our faculty continues to do an outstanding job of developing and delivering the curriculum. For a second year in a row, they have risen to the challenge of teaching a large class of students, and we are extremely grateful for their considerable time and effort in maintaining the high-quality course work that we strive to provide. The Curriculum Development and Integration Committee (CDIC)—Adrian Krainer (Chair), Z. Josh Huang, David Jackson, Leemor Joshua-Tor, and Nicholas Tonks—continues to carefully monitor and develop the curriculum. In addition to the outstanding course instructors and guest lecturers from within the Laboratory, our courses also continue to attract an impressive array of guest lecturers from other institutions.

Recruiting Efforts

Recruitment for the graduate program's 2009 class and our Undergraduate Research Program (URP) of 2009 was once again managed by Ms. Dawn Meehan, the School's Director for Admissions and Student Affairs. As in years past, Dawn traveled the length and breadth of the country, representing CSHL and WSBS. The table below details recruitment fairs and conferences in which we have participated, together with the names of faculty, students, and administrators who represented WSBS on these occasions. To further raise awareness of our programs, we also mailed more than 15,000 letters to colleges and universities in the United States and abroad.

Interinstitutional Academic Interactions

It is important to bear in mind that many of the graduate students who pursue their thesis research at CSHL are not in the WSBS graduate program. Indeed, a large percentage of students are from Stony Brook University (SBU), via a program established by CSHL and SBU more than 30 years ago. WSBS provides an on-site "home" for these students, helps to ensure that they feel part of the CSHL community, and assists them with the complexities of performing doctoral research away from their parent institutions. The following students, listed in the box below, joined us this year.

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS

Student	CSHL research mentor	Affiliation and program
Ying Cai	Richard W. McCombie	Stony Brook, Biomedical Engineering
Deblina Chatterjee	Adrian R. Krainer	Stony Brook, Molecular and Cellular Biology
Shipra Das	Adrian R. Krainer	Stony Brook, Genetics
Mathangi Ramesh	Nicholas Tonks	Stony Brook, Molecular and Cellular Biology
Yabai Song	Scott Powers	Stony Brook, Applied Math and Statistics
Yi Su	Scott Powers	Stony Brook, Applied Math and Statistics
Ruei-Ying Tzeng	Adrian R. Krainer	Stony Brook, Genetics
Ming Yang	Nicholas Tonks	Stony Brook, Biochemistry and Structural Biology

2008 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date	WSBS representatives/titles
Rice University: Biosciences Fair	Rice University	January 16	Dawn Meehan, Director of Admissions and Student Affairs
Spelman College: Graduate School and Summer Opportunities Fair	Spelman College	January 16	information sent for distribution
Wellesley College: Women in Science Fair	Wellesley College	February 27	information sent for distribution
22nd Symposium of The Protein Society Graduate School Fair	San Diego, California	July 20	Michael Pautler, Graduate Student
University of Puerto Rico: Minority Access for Research Careers (MARC) Program Information Session	University of Puerto Rico	September 10	Paloma Guzzardo, Graduate Student
University of Guelph, Canada: Information Session	University of Guelph, Canada	September 11	Joseph Calarco, Graduate Student; Michael Pautler
Universidad Interamericano de Puerto Rico: Information Session	Universidad Interamericano de Puerto Rico	September 11–12	Paloma Guzzaardo
University of Toronto: Information Session	University of Toronto	September 12	Joseph Calarco, Michael Pautler
XVIII Undergraduate Research Symposium: NSF/ UMET Model Institutions for Excellence Project	San Juan, Puerto Rico	September 12–13	Paloma Guzzardo
Georgetown University: Information Session	Georgetown University	September 13	information sent for distribution
Recruit in Canada: Graduate School Fair	Toronto, Canada	September 13	Joseph Calarco, Michael Pautler
Hunter College: Minority Access for Research Careers (MARC) Program Information Session	Hunter College	September 17	Dr. David Jackson, Professor; Dawn Meehan
Massachusetts Institute of Technology: Career Fair	Massachusetts Institute of Technology	September 18	Eugene Plavskin, Graduate Student; Hassana Oyibo, Graduate Student
Massachusetts Institute of Technology: Information Session	Massachusetts Institute of Technology	September 19	Eugene Plavskin; Hassana Oyibo
Cornell University: Information Session	Cornell University	September 23	Fred Rollins, Graduate Student, Amy Rappaport, Graduate Student
Colgate University: Information Session	Colgate University	September 23	Eugene Plavskin; Petr Znamenskiy, Graduate Student
The Johns Hopkins University: Information Session	The Johns Hopkins University	September 24	Jeremy Wilusz, Graduate Student
Cornell University: Graduate and Professional School Day	Cornell University	September 24	Fred Rollins, Amy Rappaport
University of Maryland, Baltimore County: Meyerhoff Scholarship Program Visit	University of Maryland, Baltimore County	September 29	Keisha John, Graduate Student
Big 10+ Graduate School Expo	Purdue University	September 29	Dawn Meehan
Notre Dame University: Information Session	Notre Dame University	September 30	Kyle Honegger, Graduate Student
Northwestern University: Information Session	Northwestern University	October 1	Kyle Honegger
University of California, Berkeley: Graduate School Fair	University of California, Berkeley	October 8–9	Dan Chitwood, Graduate Student
Society for Advancement of Chicanos and Native Americans in Science (SACNAS)	Salt Lake City, Utah	October 9–12	Dawn Meehan
California Institute of Technology: Career Fair	California Institute of Technology	October 15	David Simpson, Graduate Student
Washington University, St. Louis: Information Session	Washington University, St. Louis	October 16	Dr. Alyson Kass-Eisler, Curriculum Director
Emory University: Information Session	Emory University	October 20	Jeremy Wilusz
Duke University: Graduate and Professional School Day	Duke University	October 22	Joshua Sanders, Graduate Student
Yale University: Information Session	Yale University	October 22	Dr. Robert Lucito, Assistant Professor; Dawn Meehan
Emory University: Graduate Schools Fair	Emory University	October 23	Marek Kudla, Graduate Student
Harvard University: Information Session	Harvard University	October 23	Dr. Robert Lucito, Dawn Meehan
Historically Black Colleges and Universities, Undergraduate Program (HBCU–UP): National Research Conference	Atlanta, Georgia	October 23–25	Maria Pineda, Graduate Student
Annual Biomedical Conference for Minority Students (ABRCMS)	Orlando, Florida	November 5–8	Paloma Guzzardo; Dr. Adrian Krainer, Professor; Dawn Meehan; Maria Pineda; Hassana Oyibo
University of California, Santa Barbara: Graduate and Professional School Day	University of California, Santa Barbara	November 6	Megan Bodnar, Graduate Student
Princeton University: Information Session	Princeton University	November 6	Colin Malone, Graduate Student
Princeton University: Graduate and Professional School Fair	Princeton University	November 7	Colin Malone
California Forum for Diversity for Graduate Education: Graduate School Fair	University of California, Berkeley	November 8	Megan Bodnar
Barry University, Minority Access for Research Careers (MARC) Program: Information Session	Barry University	November 14	Maria Pineda
University of Pennsylvania: Bioengineering/ Biotechnology Virtual Career Fair	University of Pennsylvania	November 13– December 15	Virtual Fair
Sigma Xi Annual Conference and Research Symposium	Washington, D.C.	November 20–23	Amy Leung, Graduate Student
Vassar College: Genetics and Bioinformatics Course Visit and Information Session	Cold Spring Harbor Laboratory	November 21	Dr. Robert Lucito; Dawn Meehan; Dr. Doreen Ware, Assistant Professor; Jeremy Wilusz

A Major Change to the Graduate Student Seminar Series

In the summer of 2008, a suggestion from the SBU representative to the School's Executive Committee, Jane Lee-Osborne, prompted us to try a new format—the Graduate Student Symposium. Instead of meeting weekly, the graduate students now meet three times each year to hear talks and view posters from their colleagues. Senior students present their research in formal talks, whereas the junior students present posters in a more relaxed atmosphere. The first Symposium was held on August 28th at the Laboratory's Genome Research Center in Woodbury. The students and faculty in attendance found it to be a great event, and the feedback generated from an online survey indicated that the new format was a huge improvement over the weekly meetings. The Symposium is open to the entire Laboratory community, but it is run largely by the students themselves. We are grateful to the two student Chairs of the Symposium—Jane Lee-Osborne (SBU) and Jeremy Wilusz (WSBS)—for their dedication and hard work in helping to make this experiment a success.

Graduate Student and Postdoctoral Fellow Departures

With each year come not only new arrivals, but also departures. The following graduate students and postdoctoral fellows departed from the Laboratory during 2008:

Postdoctoral Fellows

Anindya Bagchi	Yoshitaka Hippo	Namiko Nagasawa	Khalid Siddiqui
Martin Bayer	Masateru Hiramoto	Fabio Nogueira	Matthew C. Smear
Jennifer E. Bestman	Hailan Hu	Joaquin Piriz	Andrew D. Smith
Daniel J. Bouyer	Claudia Jurgensen	Eleonore A. Real	Smita Srivastava
Julius F. Brennecke	Helmut Kessels	Seongho Ryu	Rajika Thakar
James A. Demas	Jianli Li	Lucio M. Schiapparelli	Vatsala Thirumalai
Ross A. Dickins	Susana Q. Lima	Rebecca Schwab	Dmitry Tsigankov
Juan M. Encinas	Akira Mamiya	Camile P. Semighini	Liang Wang
DingCheng Gao	Kevin J. Mc Donnell	Jihane Serkhane	Shouzhen Xia
Mengjuan Guo	Masayoshi Murakami	Pranav K. Sharma	Ning Long Xu
Haiyan He	Elizabeth P. Murchison	Wan Hua Shen	Hong Yang

Graduate Students

Claire M. Biot	Shu-Ling Chiu	Sarahjane M. Locke	Despina Siolas
Rebecca Bish	Monica Dus	Daniel J. Nolan	Deepika Vasudevan
Kasandra L. Burgos	Angélique Girard	Marissa E. Nolan	Wei Wei
Yen-Yu T. Chen	Christopher Harvey	Cristian Papazoglu-Statescu	Hatim A. Zariwala
Yi-Chun Chen	Ivan (Shun) Ho		Chaolin Zhang

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. In 2008, Lincoln Stein stepped down from the committee and was replaced by Linda Van Alest. We are grateful for the insights and advice that Lincoln brought to the Committee as a member for 5 years. As happens each year, there was also turnover among the student representatives. WSBS representative Eyal Gruntman completed his term and will be replaced by Eugene Plavskin. SBU representative Jane Lee-Osborne also completed her term and was replaced by Xiaoyun Wu. We are thankful for their honest and thoughtful advice. I wish to also thank faculty members Terri Grodzicker, W. Richard McCombie, Alea A. Mills, David L. Spector, and Nicholas Tonks, who continued to serve on the Executive Committee.

External Advisory Committee

An outstanding External Advisory Committee (EAC) guides the School. This EAC comprises Dr. Keith Yamamoto (Chair), Professor and Executive Vice Dean at the School of Medicine, University of California, San Francisco; Dr. Victor Corces, Professor, Department of Biology, Emory University, and Howard Hughes Medical Institute (HHMI) Professor; Dr. Gail Mandel, Senior Scientist, Vollum Institute, Oregon Health and Science University, and HMMI Investigator; Dr. Marguerite Mangin, Academic Programs Director and Senior Research Associate, The Rockefeller University; Dr. Barbara Meyer, Professor of Genetics and Development, University of California, Berkeley, and HHMI Investigator; and Dr. Frank Solomon, Professor, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology. The members of the Committee are noted for their leadership in graduate education in the biological sciences and are recognized leaders in their fields of research. During July 10–11, 2008, members of the EAC visited the School to review our progress since their last visit in spring 2006. As usual, the team met with students, faculty, and administrators and the overall feeling was that the School and its unique philosophy was indeed working. The EAC's continued commitment to the success of the Watson School has been irreplaceable—thank you so much!

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 CSHL an eminent researcher and educator to give the Gavin Borden Lecture, which is dedicated to the graduate students at the laboratory. Dr. Tania Baker—E.C. Whitehead Professor of Biology at the Massachusetts Institute of Technology and Howard Hughes Medical Institute Investigator—was this year's Gavin Borden Fellow. Her lecture *Remodeling Proteins and the Proteome by AAA+ Unfolding Machines* was thoroughly enjoyed and evoked many questions from the audience. In addition, Tania shared her experiences as a scientist during dinner as well as at a more structured roundtable discussion with the students the following day.

The Watson School Continues to Benefit from Generous Benefactors

I am happy to report on the establishment of the new "Gonzalo Río Arronte Fellowship" to be held by a qualified Mexican graduate student at the WSBS. Candidates for the fellowship will be preselected by the Universidad Nacional Autónoma de México and the Fundación Gonzalo Río Arronte and presented to the WSBS Admissions Committee, which will make the final decision on admission. The fellow will be expected to have a role in the development of Genomic Sciences in Mexico upon completion of his or her graduate and postdoctoral training.

We are extremely grateful for the generous donors, whose one-time gifts or continued support made our 2008 programs possible, including The Banbury Fund, The Arnold and Mabel Beckman Foundation, Mr. and Mrs. Richard M. Cashin, Mr. and Mrs. Landon Clay, Mr. Curt Engelhorn, The William Stamps Farish Fund, Lorraine G. Grace Fund, Dr. Mark Hoffman, Annette Kade Charitable Trust, Mr. and Mrs. Robert D. Lindsay, Mr. and Mrs. David L. Luke III, Mr. and Mrs. William R. Miller, and The Rathmann Family Foundation.

Student Achievements

WSBS students continue to impress us all of their accomplishments. They publish their research findings in prestigious international journals and obtain fellowships to pursue their research interests. Our students have published more than 120 papers to date, a remarkable accomplishment for a School in its tenth year.

2008 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS

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Brennecke, J., *__Malone, C.D.__, *Aravin, A.A., Sachidanandam, R., Stark, A., and Hannon, G.J. 2008. An epigenetic role for maternally inherited piRNAs in transposon silencing. *Science* **322:** 1387–1392.

Burgess, D.J., Doles, J., Zender, L., Xue, W., Ma, B., McCombie, W.R., Hannon, G.J., Lowe, S.W., and Hemann, M.T. 2008. Topoisomerase levels determine chemotherapy response *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci.* **105:** 9053–9058.

Chen, J.F., **Murchison, E.P.**, Tang, R., Callis, T.E., Tatsuguchi, M., Deng, Z., Rojas, M., Hammond, S.M., Schneider, M.D., Selzman, C.H., Meissner, G., Patterson, C., Hannon. G.J., and Wang, D.Z. 2008. Targeted deletion of Dicer in the heart leads to dilated cardiomyopathy and heart failure. *Proc. Natl. Acad. Sci.* **6:** 2111–2116.

Chiu, S.L., Chen, C.M., and Cline, H.T. 2008. Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function *in vivo*. *Neuron* **58:** 708–719.

Czech, B., *__Malone, C.D.__, *Zhou, R., Stark, A., Schlingeheyde, C., **Dus, M.**, Perrimon, N., Kellis, M., Wohlschlegel, J.A., Sachidanandam, R., Hannon, G.J., and Brennecke, J. 2008. An endogenous small interfering RNA pathway in *Drosophila*. *Nature* **453:** 798–802.

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Erlich, Y., Mitra, P.P., Delabastide, M., McCombie, W.R., and Hannon, G.J. 2008. Alta-cyclic: A self-optimizing base caller for next-generation sequencing. *Nat. Methods* **5:** 679–682.

Ewald, R.C., Van Keuren-Jensen, K.R., Aizenman, C.D., and Cline, H.T. 2008. Roles of NR2A and NR2B in the development of dendritic arbor morphology *in vivo*. *J. Neurosci*. **28:** 850–861.

Girard, A. and Hannon, G.J. 2008. Conserved themes in small-RNA-mediated transposon control. *Trends Cell Biol.* **18:** 136–148.

Gruntman, E., Qi, Y., Slotkin, R.K., Roeder, T., Martienssen, R.A., and Sachidanandam, R. 2008. Kismeth: Analyzer of plant methylation states through bisulfite sequencing. *BMC Bioinformatics* **9:** 371–384.

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Harvey, C.D., Ehrhardt, A.G., Cellurale, C., Zhong, H., Yasuda, R., Davis, R.J., and Svoboda, K. 2008. A genetically encoded fluorescent sensor of ERK activity. *Proc. Natl. Acad. Sci.* **105:** 19264–19269.

Hromádka, T., DeWeese, M.R., and Zador, A.M. 2008. Sparse representation of sounds in the unanaesthetized auditory cortex. *PLoS Biol.* **6:** e16.

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Tam, O.H., Aravin, A.A., Stein, P., **Girard, A.**, **Murchison, E.P.**, Cheloufi, S., Hodges, E., Anger, M., Sachidanandam, R., Schultz, R.M., and Hannon, G.J. 2008. Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes. *Nature* **453:** 534–539.

Wilusz, J.E., Freier, S.M., and Spector, D.L. 2008. 3′ end processing of a long nuclear-retained non-coding RNA yields a tRNA-like cytoplasmic RNA. *Cell* **135:** 919–932.

Wu, Y., **Bolduc, F.V.**, Bell, K., Tully, T., Fang, Y., Sehgal, A., and Fischer, J.A. 2008. A *Drosophila* model for Angelman Syndrome. *Proc. Natl. Acad. Sci.* **105:** 12399–12404.

Zhou, R., Hotta, I., **Denli, A.M.**, Hong, P., Perrimon, N., and Hannon, G.J. 2008. Comparative analysis of Argonaute-dependent small RNA pathways in *Drosophila*. *Mol. Cell* **32:** 592–599.

*Authors contributed equally to the work.

WSBS Family Events

Gayatri Sara Pai Stein

Finally, I am pleased to announce that this was a great year for the WSBS students, faculty, and administration because we celebrated some wonderful personal occasions. Shraddha Pai (entering class of 2004) and her husband Professor Lincoln Stein became parents when they welcomed Gayatri Sara, 6 lbs, 13 oz, on October 11. I also welcomed my own little bundle of joy—Avery Elyssa Madison was born on August 9.

Wei Wei (entering class of 2003 and 2008 graduate), Yaniv Erlich (entering class of 2006) Patrick Finigan (entering class of 2005), and Monica Dus (entering class of 2003 and 2008 graduate) all got married in 2008, and Allison Blum (entering class of 2002) and Katie Liberatore (entering class of 2008) both became engaged. Our congratulations and best wishes to all of them.

Leemor Joshua-Tor
Professor and Dean

SPRING CURRICULUM

Specialized Disciplines Courses

Due to unusual circumstances and scheduling issues, one of the Specialized Disciplines course from the fall 2007 term, *Cellular Structure and Function*, was taught in spring 2008. The class was taught as a 1-week intensive course and was universally enjoyed.

Cellular Structure and Function

FUNDED IN PART BY **The Mary D. Lindsay Lectureship, The Sigi Ziering Lectureship, The Martha F. Gerry Visiting Lectureship**

INSTRUCTORS **Linda Van Aelst (Lead)**
Raffaella Sordella

GUEST INSTRUCTORS **David L. Spector**
Bruce Stillman

VISITING LECTURERS **Michael Caplan,** Yale University
Gregg Gundersen, Columbia University
Andrea McClatchy, Massachusetts General Hospital
Conly Rieder, Wadsworth Laboratory
Sandy Simon, The Rockefeller University
Marc Symons, The Feinstein Institute for Medical Research

With the complete genome sequence available for many organisms, there is now an increasing emphasis on understanding the function of the gene products. This understanding requires an increasing appreciation of the structure and function of the cell as well as the dynamic associations within the cell. This course provided a basic overview of the structural and functional organization of cells, with particular emphasis on cellular compartmentalization and communication. Topics of focus included the cytoskeleton, cell adhesion and signaling, membrane transport, gene expression, and nuclear organization. In addition, the course provided insight into the basic toolbox of the cell biologist of the 21st century.

Topics in Biology

ARRANGED BY **Alyson Kass-Eisler** and **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 7-day courses at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the Cold Spring Harbor Laboratory Lecture Courses held each summer at the Banbury Conference Center. In Spring 2008, there were two such courses: *Immunology* and *Fundamental Concepts in Statistics*.

Immunology

Attended by the entering classes of 2004 and 2007

INSTRUCTOR	**Michael Dustin,** New York University
VISITING LECTURER	**Joel Earnst,** New York University
TEACHING FELLOWS	**Amelie Collins,** New York University **Gabriel Victora,** New York University **Serre-Yu Wong,** New York University

Immunology is an interdisciplinary field that focuses on understanding the mechanisms by which multicellular organisms defend themselves against external threats of microbial aggression and internal threats associated with genetic instability and cellular transformation. The course focused on the unique elements of the innate immune system and the adaptive immune system. Innate immunity defends against microbes by recognizing evolutionarily conserved molecular patterns. The adaptive immune system has enormous flexibility in molecular recognition, but it can also target itself to cause autoimmune diseases.

The course consisted of morning faculty presentations, afternoon readings of primary research papers and evening discussions of the research papers and related issues. The faculty presentations were in a "chalk talk" format, with questions encouraged. The afternoon reading period provided an opportunity for students to read two selected papers and related background information, teaching assistants (TAs) available for questions. The evening discussions were directed by the TAs and provided the starting point for probing intellectual and practical questions in immunology research, venturing beyond the material presented in the papers into related issues and current research. The TAs also gave short research presentations to introduce students to their particular interests and three areas of current immunology research. The course, which ran March 3–7, was developed and presented largely by Dr. Dustin, who taught at the School for the first time.

Immunology course participants: (*Front row, left to right*) Joshua Sanders, Kyle Honegger, Amelie Collins, Shraddha Pai, Megan Bodnar, Michael Dustin, Ralph Burgess. *(Middle row*, *left to right*) Joel Ernst, Garbriel Victora, Serre-Yu Wong, Oliver Fregoso, Xiaoyun Wu, Hassana Oyibo, Marek Kudla, Zhenxun Wang, Maria Pineda, Keisha John. (*Back row, left to right*) Galen Collins, Petr Znamenskiy, Paloma Guzzardo, Eugene Plavskin, David Simpson, Daniel Chitwood, Joseph Calarco, Michael Pautler. (Not shown: Saya Ebbesen.)

Fundamental Concepts in Statistics

Attended by the entering classes of 2003 and 2004

INSTRUCTOR	**Martina Bremmer,** San Jose State University
GUEST LECTURERS	**Robert Martienssen,** Cold Spring Harbor Laboratory **Madhu Mazumdar,** Weill Cornell Medical College
TEACHING FELLOWS	**Tilman Achberger,** Purdue University **Gayla Olbricht,** Purdue University

Statistics and mathematics have become an integral part of research in many areas of biology. New technology leads to large amounts of data produced in the fields of genomics, ecology, and epidemiology. The statistical methods required to analyze the data evolve and adapt constantly with the changing demands. However, the underlying principles of statistical analysis remain the same.

This course focused on the fundamental statistical concepts used in the analysis of biological data. Emphasis was placed on statistical reasoning rather than specific formulas and computations. However, examples of statistical applications were discussed and students were asked to apply the methods they learned to real data sets using software. The major statistical concepts introduced in this course included descriptive statistics, statistical inference, sampling and sampling distributions, correlation, hypothesis testing, experimental design, regression, and ANOVA.

The course integrated lectures by the instructor with directed reading and interpretation of statistical analyses in publications by the students. It was accompanied by lab sections in which the students were instructed on conducting simple statistical analyses using software. In addition, invited speakers covered special topics in the application of statistical methodology to the biological sciences. The course ran from Sunday to Saturday, June 8–14, and was organized and largely taught by Martina Bremmer. This relatively new WSBS course was highly anticipated by the students who overall thought that it was one of the most useful and best organized courses they had taken.

Fundamental Concepts in Statistics course participants: (*Back row, left to right*) Tilman Achberger, Colin Malone, Robert Martienssen, Martina Bremer, Oliver Tam, Patrick Finigan, Hiroshi Makino, Amy Rappaport, Frederick Rollins, Wen Xue, Jeremy Wilusz, Claudio Scuoppo, Gayla Olbricht. (*Front row, left to right*) Wanhe Li, Sarahjane Locke, Katherine McJunkin, Amy Leung. (Not shown: Yaniv Erlich, Eyal Gruntman, Madhu Mazumdar.)

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Amanda McBrien (Lead)**
Jennifer Cutillo
Brian Lang
Jermel Watkins
Lauren Weidler

ADMINISTRATOR **Carolyn Reid**

As science plays an increasing part 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 each week for 12 weeks. In the initial weeks, the Dolan DNA Learning Center instructors taught the Watson School students the didactic process; it was not until the fifth week that the graduate students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty the opportunity to get to know 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 and members of the School's Executive Committee attend the talks and give individual feedback to students on their presentations. This year, 20 WSBS faculty members and one research assistant professor served as rotation mentors to our largest class to date, some mentoring more than one student.

ROTATION MENTORS

Grigori Enikolopov
Hiro Furukawa
Gregory Hannon
David Jackson
Leemor Joshua-Tor
Adam Kepecs
Adrian R. Krainer
Scott Lowe
Robert Martienssen
Alea A. Mills
Senthil K. Muthuswamy
Raffaella Sordella
David L. Spector
Bruce Stillman
William Tansey
Marja Timmermans
Lloyd Trotman
Glenn Turner
Linda Van Aelst
Matt Vaughn
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, The William Stamps Farish Lectureship**

INSTRUCTORS
Gregory Hannon (Lead)
Alexander Gann
David Jackson
Leemor Joshua-Tor
Scott Lowe
Glenn Turner

GUEST LECTURERS
Josh Dubnau
Grigori Enikolopov
Hiro Furukawa
Alexei Koulakov
Robert Martienssen
Raffaella Sordella
Bruce Stillman

VISITING LECTURERS
Thomas Clandinin, Stanford University
Elizabeth Lacy, Memorial Sloan-Kettering Cancer Center
William Merrick, Case Western Reserve University
Charles Sherr, St. Jude Children's Research Hospital

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 curriculum, students (1) acquired a broad base of knowledge about the biological sciences, (2) learned the scientific method, and (3) learned how to think critically. This course consisted of six biweekly modules, each with a different theme. Each week, students read an assigned set of research articles, and at the end of the module, they provided written answers to a problem set that guided them through several of the articles. Twice weekly, students attended lectures related to the week's topic that included concepts and fundamental information as well as experimental methods. During each week, the students met among themselves to discuss the assigned papers not covered by the problem set. Each week, students spent an evening discussing the assigned articles with faculty. The module topics for this course were as follows:

Module 1 Gene Expression
Module 2 Cell Proliferation and Cancer
Module 3 Signaling in Development
Module 4 Neuroscience
Module 5 Macromolecular Structure and Function
Module 6 NIH 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	**Alea A. Mills (Lead)** **Arne Stenlund** **David J. Stewart**
TEACHING ASSISTANTS	**Christopher Faehnle** **Anthony Mazurek**
GUEST LECTURERS	**Walter Goldschmidts** **Jan A. Witkowski**
VISITING LECTURERS	**Robert Charrow,** Greenberg Traurig, LLP **Boyana Konforti,** Editor, *Nature Structural & Molecular Biology* **Maddy deLeone,** The Innocence Project **Susan McConnell,** Stanford University **Philip Reilly,** former CEO, Interleukin Genetics **Michael Rogers,** The Practical Futurist, MSNB

The 2008 *Scientific Exposition and Ethics* (SEE) core course content remained essentially the same as in 2007, but the composition of the instruction team changed: Alea Mills, who has taught the class for the past 3 years, became the lead instructor, taking over from Jan Witkowski, who had been a part of the course since its inception. Jan stepped down as an instructor to focus on his other interests, but we were fortunate to have his continued participation in the course as a guest lecturer. Drs. Arne Stenlund and David Stewart came on board this year for the first time as course instructors and were very well received. They also personally enjoyed the teaching experience and learned a great deal from the wonderful guests who visit the course each year. The faculty instructors were again joined by two postdoctoral fellows, Christopher Faehnle and Anthony Mazurek, as TAs, a format to which our students have taken very well.

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 instructors with 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 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 to be integral parts of scientific research.

Research Topics

ORGANIZERS	**Kimberley Geer** **Alyson Kass-Eisler**

This core course provided students with an in-depth introduction to 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 sem-

inars, together with the annual fall Laboratory In-House Symposium, provided students with a basis for selecting laboratories in which to do rotations. The weekly speakers were

Gurinder (Mickey) Atwal
Josh Dubnau
Mikala Egeblad
Grigori Enikolopov
Hiro Furukawa
Thomas Gingeras
Gregory Hannon
Z. Josh Huang
David Jackson
Leemor Joshua-Tor
Adam Kepecs
Alexei Koulakov
Adrian R. Krainer
Bo Li
Zachary Lippman
Scott Lowe
Robert Lucito
Robert Martienssen
W. Richard McCombie
Alea A. Mills
Partha Mitra
Pavel Osten
Darryl Pappin
Scott Powers
Jacek Skowronski
Raffaella Sordella
David L. Spector
Arne Stenlund
Bruce Stillman
Marja Timmermans
Lloyd Trotman
Glenn Turner
Linda Van Aelst
Doreen Ware
Michael Wigler
Anthony Zador
Michael Q. Zhang
Yi Zhong

SPECIALIZED DISCIPLINES COURSES

The content of the three *Specialized Disciplines in Biology* (SD) courses—*Genetics, Systems Neuroscience* and *Cellular Structure and Function*—has largely remained the same. The SD course in genetics was renamed *Genetics and Genomics*, and lead instructor Josh Dubnau was joined by genomics expert Thomas Gingeras as an instructor this year. The SD course *Systems Neuroscience* was taught by lead instructor Anthony Zador and instructor Adam Kepecs, and the SD course *Cellular Structure and Function* was taught by lead instructor Linda Van Aelst and instructor Raffaella Sordella.

Genetics and Genomics

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 **Josh Dubnau (Lead)**
Thomas Gingeras

GUEST LECTURER **Clint Whipple, Postdoctoral Fellow,** Jackson laboratory

VISITING LECTURERS **Bambos Kyriacou,** University of Leicester, United Kingdom
Marla Sokolowski, University of Toronto

This course placed modern human genetics and genomics into the context of classical organismal genetics. History, perspective, and technique were described around four levels of analysis: naturally occurring variation, genome evolution, genetic screens, and gene epistasis. How do gene mutations help to define biological processes? How are more complex traits genetically dissected into simpler (underlying) components? What concepts and techniques are used to organize genes into pathways and networks? How are genes mapped, cloned, and engineered to identify functional domains of proteins? What gene variation exists in natural populations? What are the functional consequences of gene variation? How is it detected? How are genomes organized and coordinately regulated? How can genomic information be cataloged, organized, and mined? These questions and concepts were fleshed out using examples from the literature.

Systems Neuroscience

FUNDED IN PART BY **The George W. Cutting Lectureship, The Klingenstein Lectureship, The William R. Miller Lectureship**

INSTRUCTORS **Anthony Zador (Lead)**
Adam Kepecs

GUEST INSTRUCTORS **Alexei Koulakov**
Glenn Turner

VISITING LECTURER **Mayank Mehta,** Brown University

This course provided an overview of key aspects of neuroscience. The emphasis was on spanning levels: How can we go from molecules through cells and circuits to behavior? There were three main components to the class: lectures, problem sets, and a final project. The last week of class was spent on the final project, in which the students found and presented a neuroscience paper that spans levels, for example, from the molecular to the synaptic or from the circuit level to the behavioral.

Cellular Structure and Function

FUNDED IN PART BY **The Mary D. Lindsay Lectureship, The Sigi Ziering Lectureship, The Martha F. Gerry Visiting Lectureship**

INSTRUCTORS **Linda Van Aelst (Lead)**
Raffaella Sordella

GUEST INSTRUCTORS **David L. Spector**
Bruce Stillman

VISITING LECTURERS **Michael Caplan,** Yale University
Gregg Gundersen, Columbia University
Elizabeth Miller, Columbia University
Conly Rieder, Wadsworth Laboratory
Sandy Simon, The Rockefeller University
Marc Symons, The Feinstein Institute for Medical Research

With the complete genome sequence available for many organisms, there is now an increasing emphasis on understanding the function of the gene products. This understanding requires an increasing appreciation of the structure and function of the cell as well as the dynamic associations within the cell. This course provided a basic overview of the structural and functional organization of cells, with particular emphasis on cellular compartmentalization and communication. Topics of focus included the cytoskeleton, cell adhesion and signaling, membrane transport, gene expression, and nuclear organization. In addition, the course provided insight into the basic toolbox of the cell biologist of the 21st century.

POSTDOCTORAL PROGRAM

PROGRAM DIRECTOR **Nicholas Tonks**

PROGRAM ADMINISTRATOR **Alyson Kass-Eisler**

Cold Spring Harbor Laboratory (CSHL) is proud of its rich tradition in postdoctoral education and has a deep commitment to the postdoctoral experience. CSHL has long been recognized as a place for nurturing young scientists, with postdoctoral researchers being an integral part of the discovery process. Approximately 135 postdoctoral fellows work in the labs of 43 principal investigators at the Laboratory. Current fields of research expertise include genetics; molecular, cellular, and structural biology; neuroscience; cancer; plant biology; bioinformatics and genomics; and quantitative biology. The postdoctoral community at CSHL is diverse and international, arriving at the Laboratory with many different backgrounds and with needs that change during the course of the postdoctoral years. The Postdoctoral Program Office works closely with the fellows and Laboratory administration to coordinate and organize educational and career development activities. Dr. Alyson Kass-Eisler, Postdoctoral Program Officer, and Dr. Nicholas Tonks, Scientific Director of the Postdoctoral Program, provide day-to-day support for the postdoctoral program, which is overseen by the Dean of the Watson School, Dr. Leemor Joshua-Tor.

The Dean of the WSBS hosts "Dean's teas" for trainees who have recently arrived at CSHL, with the goal of helping fellows to make the most of their postdoctoral experience here. During these sessions, postdoctoral fellows are provided information on the services available through the Postdoctoral Program Office and are given copies of the excellent books by Kathy Barker, "At the Bench: A Laboratory Navigator" and "At the Helm: A Laboratory Navigator," published by Cold Spring Harbor Laboratory Press. The Postdoctoral Program Officer meets with prospective or newly arrived fellows to inform them about the program and to answer any questions that they may have. She also works with fellows one on one in finding sources of funding and assisting with applications, and later, she assists senior postdocs with identifying career opportunities and preparing application materials. On October 30, all postdoctoral fellows were invited to attend a meeting of the CSHL Postdoctoral Association along with the postdoctoral program administration—Leemor Joshua-Tor, Nick Tonks, and Alyson Kass-Eisler. The purpose of the meeting was to bring postdoctoral fellows together to discuss ways of improving the postdoctoral experience at the Laboratory and to answer any questions or concerns about the program. A number of great ideas were discussed and we look forward to introducing some new services in the near future.

In 2008, we held a number of courses and workshops aimed at postdoctoral fellows. For example, in the spring, the Laboratory held a course on grant writing. The first part, led by Walter Goldschmidts and his CSHL Office of Sponsored Research staff, focused on an introduction to grant writing and the grant application process. During the second part of the course, CSHL faculty members William Tansey and Linda Van Aelst gave an overview of the grant review process with their presentation *True Confessions of Peer Review*. The third part of the course was an interactive writing session led by Dr. Philip Vassallo, author of *The Art of On-The-Job Writing.*

An ethics course that provided training in responsible conduct of research was also held this spring. The course included sessions on *Roles and Responsibilities Associated with Research Funding*, *Conflicts of Interest*, *Selling Yourself*, *Presentations—Engage Your Audience,* and *Requirements on Statistical Analysis in Publications*.

Laboratory President Dr. Bruce Stillman holds an annual "Town Hall" meeting specifically for postdoctoral fellows. This year's Town Hall meeting took place on December 17. Among the ideas that were discussed was the updating of the postdoctoral program website to enhance its usefulness as a networking tool. We will be working with a small committee of postdocs to put together suggestions for the content and layout of this enhanced site.

CSHL has been a member of a special ambassador recruitment program from Merck Research Laboratories (MRL) since 2005. This program provides postdoctoral fellows and students at CSHL an inside

connection to a scientist working at MRL. As a result of this program, a postdoctoral fellow from Dr. Bruce Stillman's laboratory, Dr. Maarten Hoek, was hired as a Research Scientist in 2007. In 2008, Dr. Jing Li returned to the Watson School and was joined by Maarten, who presented his current work in a seminar entitled "Proteomic Identification of Caspase Cleavage Events in Apoptotic Cells." In addition, nine postdoctoral fellows met with Jing and Maarten in one-on-one interviews. As a result of this program, Dr. Zuo Zhang, a postdoctoral fellow from Dr. Adrian Krainer's laboratory, joined MRL as a Research Scientist at the end of 2008. The relationship between MRL and CSHL has been extremely beneficial and we will be looking toward building additional relationships in the future.

Three years ago, the School initiated a prize for the best posters by a postdoctoral fellow and the best poster by a graduate student at the Laboratory's annual In-House Symposium. In addition to providing a forum for the postdoctoral fellows to show off their research, and potentially win a prize, it gives the entire scientific community a chance to come together and share ideas beyond the walls of their individual laboratories. It has been a great success for both the presenters and the laboratory community as a whole. Each "prize" is given in the form of an educational grant of $500.

During the past couple of years, we have increasingly widened the role of postdoctoral fellows in the curricular activities of the School. Our fellows have been involved in preparing our students for the challenging fall term curriculum by working as one-on-one tutors during the summer. Throughout the fall term, postdoctoral fellows are used as tutors for all aspects of the curriculum, providing individual and group tutoring and participating in discussion sessions. In 2007, the *Scientific Exposition and Ethics* course took further advantage of the expertise of our postdoctoral community by hiring two fellows as TAs. These two fellows proved to be an integral part of the course by providing their expertise in discussions, editing the students' written work, and critiquing oral presentations. This format was highly successful and was used again in fall 2008.

This spring, we hosted a delegation from the Galicia region of Spain. Dr. Laura Sánchez Piñón, Chancellor of Education, and Dr. Pilar Fernández González, Education Advisor, General Consulate of Spain, came to the Laboratory as part of a visit to high-profile research institutions in the United States, with the goal of gathering information to improve science in their region. Our Spanish postdocs were excited to meet with the delegation, share their experiences at CSHL, and help to introduce our best practices to Spain.

2008 delegation from Spain: (*Left to right*) Cristina Fernandez Marco, Pilar Fernández González (Education Advisor, General Consulate of Spain), Victoria Aranda Calleja, Mikel Biurrun, Yoselin Benitez Alfonso, Xavier Roca, Laura Sánchez Piñón (Chancellor of Education, Galicia, Spain), Juan Manuel Encinas, Juan Manuel Abolafia Moya, Jose Silva, Andres Canela.

Since 2003, all postdoctoral fellows and graduate students at the Laboratory have been enrolled in a special initiative of the New York Academy of Science (NYAS), called the Science Alliance. The Science Alliance for graduate students and postdoctoral fellows is a consortium of universities, teaching hospitals, and independent research facilities in the New York City metropolitan area that have formed a partnership with NYAS. The aim of the Alliance is to provide career and professional development monitoring for postdoctoral fellows and graduate students in science and engineering, through a series of live events and a dedicated web portal. In addition, the Science Alliance gives graduate students and postdoctoral fellows the opportunity to network with their peers across institutions and with key leaders in academia and industry.

To complement our local efforts, we have been involved in workshops at a national level aimed at shaping the postdoctoral experience. The Laboratory became a founding member of the National Postdoc Association (NPA) in 2004. In April 2008, Alyson Kass-Eisler attended the sixth annual meeting in Boston, Massachusetts. Topics of interest to the Laboratory's postdocs included *Creating a Dynamic Program on Responsible Conduct of Research, Providing Career Development Resources, Mentoring Tools: A Toolbox of Ideas!, Navigating Your Career...Immigration Obstacles, Ensuring PDA Sustainability and Growth, How to Assist Postdocs...Successful Fellowship,* and *NIH Pathway to Independence.*

In 2008, the WSBS also participated in the National Research Council's Assessment of Graduate Programs, which aims to provide a metric for comparing graduate programs in similar disciplines to one another. One component of this survey is the quality of postdoctoral programs. We look forward to seeing the results of this survey, which will hopefully provide another measure of the success of our program and ways in which it can be improved. The results of the survey were initially expected in 2008, but they are now set to be released in early spring 2009.

Finally, two important measures of our postdoctoral program's success are the ability of postdoctoral fellows to secure individual fellowships for their training and permanent positions at the end of their training. We are extremely proud that our postdoctoral fellows have been successful in both of these endeavors.

Current postdocs hold fellowships from a number of national, international, federal, state, and private foundations including the American Cancer Society; Brodeur Breast Cancer Foundation; Chapman Fellowship; DAAD, German Academic Exchange Service; DFG German Science Foundation; Ellison Medical Foundation; Heart and Stroke Foundation; Human Frontier Science Program; Harvey L. Karp Foundation; Lauri Strauss Leukemia Foundation; NARSAD; National Institutes of Health (NIH); New York State Department of Health, HRSB, Breast Cancer Research and Education Program; The Patterson Trust; Sass Foundation Fellowship; Sir Keith Murdock fellowship; The Andrew Seligson Memorial Clinical Fellowship for Cancer Research; The Leukemia and Lymphoma Society; The Lymphoma Research Foundation; United States Department of Agriculture; and the United States Department of Defense. Three CSHL postdoctoral fellows have also been fortunate to secure career development funding through the prestigious NIH's Pathways to Independence Award. Alexei Aravin, a postdoctoral fellow in Gregory Hannon's laboratory, was named a finalist for the New York Academy of Science's Blavatnik Award, and Erkan Karakas, from Hiro Furukawa's laboratory, received CSHL's Harvey L. Karp award. To increase the Laboratory's fellowship funding and to bring new programs to CSHL, the School will be submitting a new postdoctoral training grant to the NIH in May.

In 2008, the Laboratory's departing postdoctoral fellows went on to positions at Memorial Sloan-Kettering Cancer Center, Merck Research Laboratories, The Walter and Eliza Hall Institute of Medical Research (Australia), University of Minnesota, Columbia University, Max-Planck Institute (Germany), Louis Pasteur University (France), The Champalimaud Foundation (Portugal), and Institute of Molecular Biotechnology (Austria), to name just a few.

UNDERGRADUATE RESEARCH PROGRAM

PROGRAM DIRECTORS **David Jackson**

PROGRAM ADMINISTRATOR **Dawn Meehan**

Each summer, 20–25 undergraduates from around the world and across the country participate in the CSHL summer Undergraduate Research Program (URP). More than 720 students have participated in the URP since the program was founded in 1959.

The fundamental objective of the program is to give students an opportunity to conduct first-rate research. Participants learn about scientific reasoning, laboratory methods, theoretical principles, and scientific communication. The specific objectives of the program are to (1) give college undergraduates a taste of conducting original research at the cutting edge of science, (2) encourage awareness of the physical and intellectual tools necessary for modern biological research, (3) foster an awareness of the major questions currently under investigation in the biomedical and life sciences, and (4) promote interactions with Laboratory scientists through an immersion in the research environment.

During the 10-week program, URP participants work with CSHL senior staff members on independent research projects, specifically in the areas of cancer biology, neuroscience, plant biology, cellular and molecular biology, genetics, macromolecular structure, and bioinformatics.

URPs work, live, eat, and play among CSHL scientists. They have a very busy academic and social calendar for the summer. They attend lectures in the Goldberg Faculty Lecture Series given by CSHL faculty members and outside faculty members including, in 2008, Dr. Stanley Maloy of San Diego State University and Dr. Alfred Goldberg of Harvard University. URP students also attend a Bioinformatics Workshop Series, where they learn how to identify patterns in DNA and protein sequences and interpret them. Lectures given by CSHL faculty are specifically designed for URP students. URP participants were also invited to join Dr. and Mrs. Watson for a pizza party and Dr. and Mrs. Stillman for dinner. BBQs and pool parties, volleyball games, sailing lessons, designing the URP tee shirt, competing in the annual CSHL Petri Dish Race and Scavenger Hunt, and the ever-famous URP vs. PI volleyball match rounded out the engaging program. This year was the first that the URPs beat the faculty team in volleyball—quite an accomplishment!

Undergraduate Research Program 2008 participants in the URP tee shirt design contest: (*Front row, left to right*) Olga Minkina, Yesenia Correa, Allison Baker, Anna Gilman, Susan Shen, Tzitziki Lemus Vergara. (*Back row, left to right*) Richard Gerrard, Eric Domb, Jonathan Geisinger, Erin Jimenez, Daniel Kim, Walter Barry, Colleen Carlston, Ryan Ly, Xun Hou, Richard Jin, Forest Ray, Zandra Walton, Chris Hsiung.

At the beginning of the summer, URP students write an abstract and present a talk on their proposed research. Concluding the program in August, URP participants prepare a final report and present their results in a 15-minute talk at the URP Symposium. The following students, selected from 822 applicants, took part in the 2008 program:

Allison Baker, Dartmouth College
Advisor: **Dr. Tony Zador**
Sponsor: National Science Foundation and Department of Defense and Libby Fellowship
A role for rat auditory cortex in attention in time to auditory stimuli.

Walter Barry, Tufts University
Advisor: **Dr. Bruce Stillman**
Sponsor: William Shakespeare Fund Fellowship
Analysis of yeast replication origins via two-dimensional gel electrophoresis.

Colleen Carlston, Harvard University
Advisor: **Dr. Josh Dubnau**
Sponsor: Burroughs-Wellcome Fund Fellowship
Pavlovian conditioning of the immune system.

Yesenia Correa, Oregon State University
Advisor: **Dr. Scott Powers**
Sponsor: National Science Foundation and Department of Defense and C. Bliss Memorial Fund Fellowship
Influence of initial differentiated state of the normal cell on the final tumorigenic phenotype.

Eric Domb, Princeton University
Advisor: **Dr. Michael Zhang**
Sponsor: National Science Foundation and Department of Defense and Garfield Fund Fellowship
In silico detection of *cis*-regulatory modules.

Jonathan Geisinger, Case Western Reserve University
Advisor: **Dr. William Tansey**
Sponsor: H. Bentley Glass Fund Fellowship
Ubiquitylation and stability of an ubiquitin ligase RPC.

Richie Gerrard, University of St. Andrews
Advisor: **Dr. David Spector**
Sponsor: James D. Watson Fund Fellowship
In vivo studies of the H3K27 demethylase JmjD3.

Anna Gilman, Barnard College, Columbia University
Advisor: **Dr. Scott Lowe**
Sponsor: Joan Redmond Read Fund Fellowship
Dissecting tumor suppressor mechanisms using conditional RNA interference.

Xun Hou, Massachusetts Institute of Technology
Advisor: **Dr. Dick McCombie**
Sponsor: Former URP Fund Fellowship
Identifying SNP variation of rapidly evolving genes.

Chris Hsiung, University of California, Berkeley
Advisor: **Dr. Greg Hannon**
Sponsor: Steamboat Foundation Fellowship
Fishing for small RNAs in Argonaute complexes.

Erin Jimenez, University of California, Los Angeles
Advisor: **Dr. Dave Jackson**
Sponsor: National Science Foundation and Department of Defense and William Townsend Porter Fund Fellowship
Further defining the location of the *Abphyl2* gene by positional cloning and understanding the mechanisms controlling phyllotaxy in maize with *Abphyl2* mutants.

Richard Jin, Cornell University
Advisor: **Dr. Rob Martienssen**
Sponsor: Robert H.P. Olney Fund Fellowship
Replication initiation points in *S. pombe.*

Daniel Kim, Amherst College
Advisor: **Dr. Adam Kepecs**
Sponsor: National Science Foundation and Department of Defense and Emanuel Ax Fund Fellowship
Using optogenetics to study network mechanisms of θ oscillations in the hippocampus.

Tzitziki Lemus Vergara, Universidad Nacional Autonoma de Mexico
Advisor: **Dr. Doreen Ware**
Sponsor: Former URP Fund Fellowship
Phylogenetics of the maize tetraploid genome.

Ryan Ly, Johns Hopkins University
Advisor: **Dr. Partha Mitra**
Sponsor: Former URP Fund Fellowship
Learning and memory in the *Drosophila* flight simulator.

Olga Minkina, Washington University, St. Louis
Advisor: **Dr. Marja Timmermans**
Sponsor: Former URP Fund Fellowship
The role of AS1/AS2 and *TAS3* in determining abaxial-adaxial leaf polarity.

Forest Ray, Hunter College
Advisor: **Dr. Alea Mills**
Sponsor: Hunter College Fund Fellowship
Tumor-derived mutations in CHD5.

Susan Shen, California Institute of Technology
Advisor: **Dr. Josh Huang**
Sponsor: Von Stade Fund Fellowship
GABA(A) receptor subcellular localization, dynamics, and function.

Zandra Walton, Amherst College
Advisor: **Dr. Senthil Muthuswamy**
Sponsor: Former URP Fund Fellowship
Scribble expression in mammary epithelial cells with low *let7c* miRNA.

PARTNERS FOR THE FUTURE

PROGRAM DIRECTOR **Yuri Lazebnik**

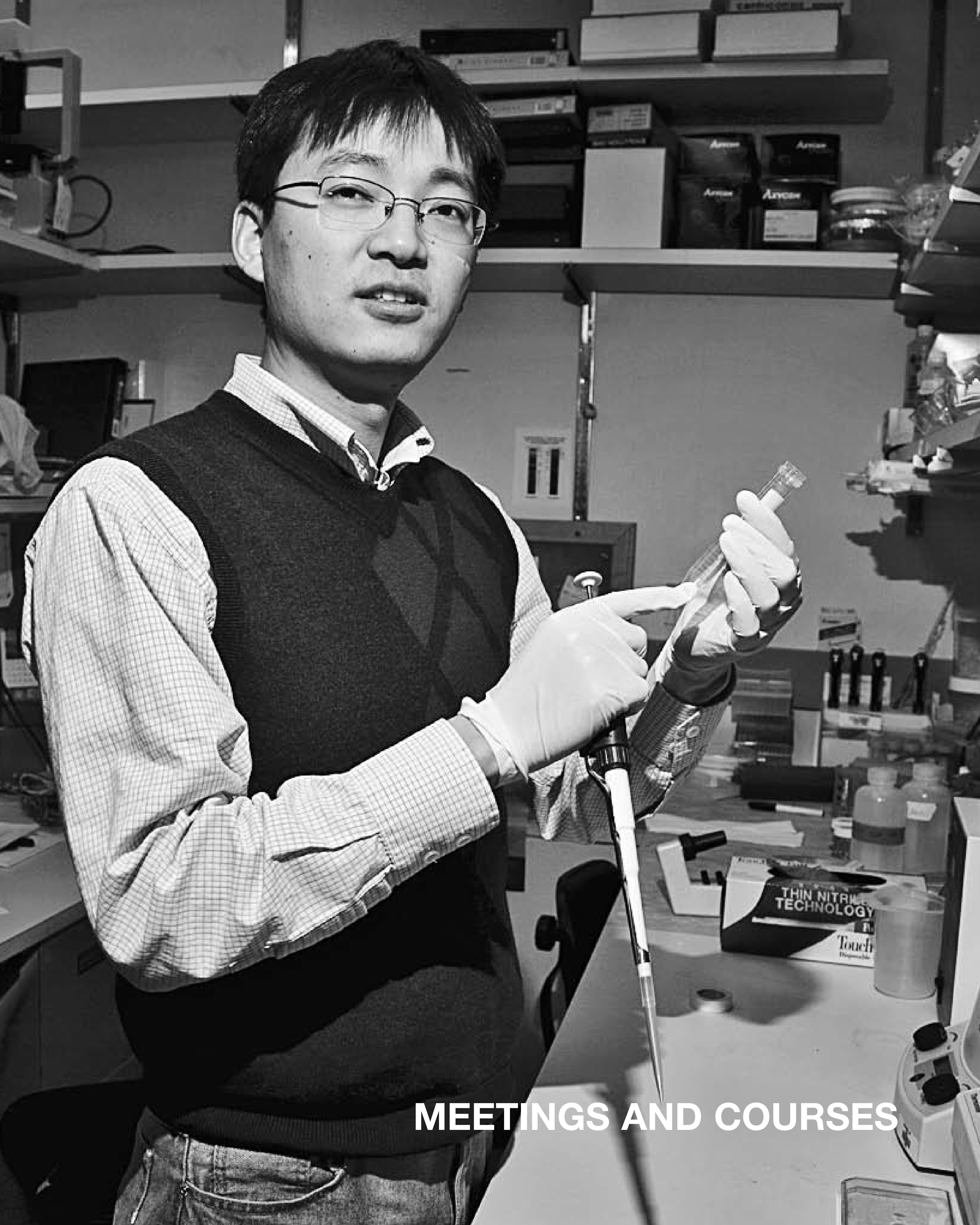
PROGRAM ADMINISTRATOR **Theresa Saia**

The Partners for the Future Program, established by Dr. James Watson in 1990, provides an opportunity for talented Long Island high school students to have hands-on experience in biomedical research at Cold Spring Harbor Laboratory. This highly competitive program is open to Long Island high school students in their junior year. Each high school science department chairperson may nominate up to three students. The top candidates are interviewed by CSHL scientists and up to ten top students are chosen to participate in the program. Students selected to the program are paired with a scientist mentor and spend a minimum of ten hours per week, September through March of their senior year, conducting original research. At the conclusion, the students present their projects to an enthusiastic audience of the students' scientific mentors and colleagues, CSHL 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 that they are exposed to day-to-day life in a lab. Interacting with scientists and support staff, the students learn to define and pursue a research goal while solving problems that may occur along the way.

The 2008–2009 Partners for the Future were chosen from among 16 semifinalist nominations and they are listed here.

Partner	High School	CSHL mentor	Laboratory
Kay Chen	Ward Melville High School	Clint Whipple	David Jackson
Josh Elkin	Cold Spring Harbor High School	Zhenyu Xuan	Michael Zhang
Jon McGinn	Farmingdale High School	Anirban Paul	Josh Huang
Elizabeth Sajewski	Cold Spring Harbor High School	Hysell Oviedo	Tony Zador
Madeline Scircco	Cold Spring Harbor High School	Morgan Xu	David Jackson
Catherina Yang	Syosset High School	Shilpi Paul	Alea Mills
Patriot Yang	Syosset High School	Doreen Ware	Doreen Ware

2008–2009 Partners for the Future participants: (*Left to right*) Jon McGinn, Kay Chen, Madeline Scircco, Elizabeth Sajewski, Josh Elkin, Patriot Yang, Catherina Yang.



MEETINGS AND COURSES

ACADEMIC AFFAIRS

The Meetings and Courses program at Cold Spring Harbor 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. More than 8000 scientists ranging from graduate students and postdoctoral fellows to senior faculty come from around the world to attend these events. A growing international program complements the main program of meetings and courses.

In 2008, 27 laboratory and lecture courses attracted more than 1230 participants (including teaching faculty, students, and technicians). These courses covered a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim of the courses is to teach students the latest advances that can be immediately applied to their own research. Courses are always being evaluated and updated to include the latest concepts and approaches. Increasingly, many courses feature a strong computational component as biology grows ever more interdisciplinary, incorporating methodologies from computer science, physics, and mathematics. A new bioinformatics course on Computational Cell Biology was started in 2008.

Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor Laboratory. Their excellence and dedication make the course program work so well. The full program of 2008 courses and instructors are listed on the following pages. We would especially like to thank Drs. Philip Andrews, Beverley Clark, Arshad Desai, Jeffrey Diamond, James Eberwine, Marc Freeman, Joshua La Baer, Frank Luca, Lincoln Stein, David Threadgill, Paul Trainor, and Bing Zhang, who all retired this year after many years of service.

Grants from a variety of sources support the courses. Core support provided through the recently renewed grant from the Howard Hughes Medical Institute remains critical to our course program. The courses are further supported by multiple awards from the National Institutes of Health (NIH) and the National Science Foundation (NSF), and additional support for individual courses is provided from various foundations and other sources. The courses also depend on equipment and reagents that are loaned or donated by a large number of companies. These are invaluable for making it possible to keep up with the latest technologies.

The Laboratory held 25 academic meetings this year that brought together almost 7000 scientists from around the world to discuss their latest research. The Symposium—now in its 73rd year—continues to be the flagship conference of the meetings program. This year's meeting on Control and Regulation of Stem Cells addressed our current understanding of the biology of pluripotent and totipotent stem cells and featured 67 talks and 178 poster presentations. Opening night speakers included Rudolf Jaenisch, Minx Fuller, Arturo Alvarez-Buylla, and Max Wicha. Irving Weissman presented the Reginald Harris lecture on "Normal and neoplastic stem cells." Elaine Fuchs enlightened a mixed audience of scientists, lay friends, and neighbors with her Dorcas Cummings lecture on "Skin stem cells," and Brigid Hogan ended the meeting with a masterful and eloquent summary. We particularly thank the Lisbon-based Champalimaud Foundation for their generous support of this year's Symposium.

G. Digby and T. Abrahamsson at lab session of Imaging Structure and Function of the Nervous System course

A prime feature of the meetings is that there are very few invited speakers. Meetings organizers select talks from abstracts that are submitted. This format ensures that the latest findings will be presented and that young scientists will have the chance to describe their work. The annual meetings on Gene Expression and Signaling in the Immune System, The Biology of Genomes, Retroviruses, Mechanisms and Models of Cancer, and the annual Symposium were all oversubscribed, and many others attracted record attendances including the meetings on Systems Biology: Global Regulation of Gene Expression; Translational Control; Axon Guidance, Synaptogenesis, and Neural Plasticity; and Molecular Genetics of Aging. New meetings on Epithelial-Mesenchymal

Transition, Personal Genomes, and Blood Brain Barrier Physiology were held this year. Many of these meetings, held on a biannual basis, have become essential for those in the field. Partial support for individual meetings is provided by grants from the NIH, NSF, foundations, and companies. Core support for the meetings program is provided by the Laboratory's corporate patrons, benefactors, sponsors, affiliates, and contributors.

The joint Cold Spring Harbor/ Wellcome Trust conference series held at the genome campus south of Cambridge, England expanded to include six meetings on Computational Cell Biology, Network Biology, Genomic Perspectives to Host-pathogen Interactions, Genome Informatics, Integrative Approaches to Brain Complexity, and Rat Genomics and Models. These conferences follow the Cold Spring Harbor model in that the majority of talks are selected from the abstracts. They attracted 559 participants.

Finally, 2008 saw the formal beginning of the Cold Spring Harbor Conferences Asia program, with the incorporation of CSH Conferences Asia as a wholly owned subsidiary. The inaugural meeting of CSH Asia's Scientific Advisory Board held in Suzhou, China in early October brought together eminent scientists from throughout Asia to advise the Laboratory on its new educational initiative in China and Asia.

The success of the very large number of meetings and courses is also due to the skilled work of many Laboratory staff and faculty who contribute their expertise, efforts, and good humor to the program.

Terri Grodzicker
Dean
Academic Affairs

David Stewart
Executive Director
Meetings and Courses Program

W. Pearson (*middle*) instructs participants during Computational and Comparative Genomics course.

C. Sherr and D. Bar-Sagi, co-organizers of the Cancer Models and Mechanisms meeting

T. Taylor and E. Bruford celebrate their joint win of the Genome Crossword.

E. Fuchs, 2008 Dorcas Cummings lecturer

73RD COLD SPRING HARBOR LABORATORY SYMPOSIUM ON QUANTITATIVE BIOLOGY

Control and Regulation of Stem Cells

May 28–June 2 465 participants

ARRANGED BY **Terri Grodzicker, David Stewart, and Bruce Stillman**
Cold Spring Harbor Laboratory

The roots of stem cell research can be traced back to classical work in embryology and regeneration performed in the 19th century. By the early 1900s, researchers in Europe had come to understand that various types of blood cells derived from a particular "stem cell," resulting in physicians' attempts to administer bone marrow by mouth to patients suffering from anemia and leukemia. Although such therapy was unsuccessful, laboratory experiments eventually demonstrated that mice with defective marrow could be restored to health with infusions into the bloodstream of marrow taken from other mice, causing physicians to speculate whether it was feasible to transplant bone marrow from one human being to another. Early work on bone marrow transplantation—effectively, a stem cell transplant—was dogged by problems of histocompatibility, but as the basis of the HLA system became increasingly understood, successful bone marrow transplantation between unrelated individuals was first demonstrated in 1973, followed by the discovery of true hematopoietic stem cells in 1978.

Stem cell research has since exploded, with the derivation of mouse embryonic stem cells in 1981, the culturing of neural stem cells as neurospheres in 1992, and the establishment of the first human embryonic stem cell lines in 1998. Around the same time as the isolation of human embryonic cells, a definitive link between stem cells and cancer was established when certain leukemias were shown to originate from hematopoietic stem cells. In the last decade, the concept of adult stem cell plasticity has gained credence, although many findings have proved to be controversial, whereas the ethical arguments for and against the use of human embryonic stem cells have been widely debated at national and international levels. Within the last 2 years, it has been reproducibly established that an embryonic stem-cell-like state, previously achieved only by somatic cell nuclear transfer into enucleated oocytes ("cloning") or by fusion with embryonic stem cells, can be induced by reprogramming of differentiated adult cells using a simple combination of key transcription factors.

B. Stillman, A. Denli

Progress in stem cell research is now extremely intense, with more than 5000 research papers on embryonic and adult stem cells being published in reputable scientific journals every year. It therefore seemed appropriate to focus the 73rd Symposium on this important and rapidly developing field, providing a unique synthesis of the exciting progress being made in the field of stem cell biology not only for the Symposia attendees, but for a wider global audience via interviews freely available on the World Wide Web and through the Symposium proceedings.

In organizing this Symposium, we relied on the assistance of Elaine Fuchs, Rusty Gage, Greg Hannon, Ron McKay, Davor Solter, and Allan Spradling for suggestions for speakers. Opening night speakers included Rudolph Jaenisch, Minx Fuller, Arturo Alvarez-Buylla, and Max Wicha, and Irving Weissman presented the Reginald Harris lecture on "Normal and neoplastic stem cells." Elaine Fuchs enlightened a mixed audience of scientists and lay friends and neighbors with her Dorcas Cummings lecture

on “Skin stem cells,” and Brigid Hogan ended the meeting with a masterful and eloquent summary.

This Symposium was attended by 465 scientists from more than 30 countries, and the program included 68 oral presentations and 177 poster presentations. We particularly thank the Lisbon-based Champalimaud Foundation for their extensive and generous support of this year’s Symposium. Additional funds to run this meeting were obtained from the National Institutes of Health. Financial support 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 Patrons* included Pfizer Inc. *Corporate Benefactors* included Amgen Inc., GlaxoSmith Kline, Merck Research Laboratories, and Novartis Institutes for BioMedical Research. *Corporate Sponsors* included Agilent Technologies, Applied Biosystems, AstraZeneca, BioVentures, Inc., Bristol-Myers Squibb Company, Genentech, Inc., Hoffmann-La Roche Inc., Invitrogen Corporation, IRX Therapeutics Inc., Kyowa Hakko Kogyo Co., Ltd., New England BioLabs, Inc., OSI Pharmaceuticals, Inc., Sanofi-Aventis, and Schering-Plough Research Institute; *Plant Corporate Associates* included Monsanto Company, and Pioneer Hi-Bred International, Inc. *Corporate Affiliates* included Affymetrix. *Corporate Contributors* included Epicentre Biotechnologies and Illumina*.* *Foundations* included Hudson Alpha Institute for Biotechnology.

PROGRAM

Introduction
R. Lehmann, *New York University School of Medicine, New York*

Germ Cells and Epigenetics
Chairperson: B. Hogan, *Duke University Medical Center, Durham, North Carolina*

Signaling in Adult Stem Cells
Chairperson: D. Melton, *HHMI, Harvard University, Cambridge, Massachusetts*

Gene Expression
Chairperson: R. Young, *Whitehead Institute/MIT, Cambridge, Massachusetts*

Reginald B. Harris Lecture: Normal and Neoplastic Stem Cells
Chairperson: I. Weissman, *Stanford University School of Medicine, California*

Cancer I
Chairperson: S. Morrison, *University of Michigan, Ann Arbor*

Pluripotency in ES Cells
Chairperson: D. Solter, *Max-Planck Institut für Immunobiologie, Freiburg, Germany*

Neural Stem Cells
Chairperson: J. Rich, *Duke University Medical Center, Durham, North Carolina*

Dorcas Cummings Lecture: Skin Stem Cells
Elaine Fuchs, *HHMI/The Rockefeller University*

E. Fuchs

C. Cormier

B. Hogan, J. Alfred

A. Surani, I. Weissman

Cancer II
Chairperson: C. Sherr, *HHMI/St. Jude Children's Research Hospital, Memphis, Tennessee*

Germ Cells and Regeneration
Chairperson: M. Fuller, *Stanford University School of Medicine, California*

Adult Stem Cells II
Chairperson: M. Buckingham, *Institut Pasteur, Paris, France*

Niches and Asymmetry
Chairperson: A. Spradling, *Carnegie Institute of Washington, Baltimore, Maryland*

Summary
B. Hogan, *Duke University Medical Center, Durham, North Carolina*

A. Spradling, J. Greve

T. Grodzicker, R. McKay

K. Zaret, R. Young

J. Botelho, D. Stewart

Walking tour

MEETINGS

PTEN Pathways and Targets

March 5–9 114 Participants

ARRANGED BY **Suzanne Baker,** St. Jude Children's Research Hospital
Carlos Cordon-Cardo, Columbia University
Pier Paolo Pandolfi, Harvard University
Ramon Parsons, Columbia University

This was the second conference on PTEN Pathways and it was enormously successful. The meeting brought together top scientists studying the PTEN/PI3K pathway using biochemical and molecular approaches as well as in vivo model systems. An exciting series of talks and poster presentations addressed the complex regulation of this signal transduction cascade and its critical role in normal development, cancer, and other pathological disease states. The conference provided an opportunity for researchers of all levels to come together to discuss their latest research findings and technical approaches toward the analysis of the PTEN pathway. Particular emphasis was placed on pathway regulation and cross-talk, human cancer pathogenesis, disease models, and therapy.

J. Watson, R. Parsons, C. Cordon-Cardo

A total of 38 investigators presented in the nine scientific sessions, with nearly 55 platform and poster presentations and 114 registered attendees. The nine platform and two poster sessions were marked by dynamic and enthusiastic exchanges of new results. In the scientific sessions, many audience members participated in the question and answer sessions, and the poster sessions were well attended.

Essential funding for the meeting was provided by the National Cancer Institute, a branch of the National Institutes of Health. Myriad Genetics, Inc. also provided a $3000 prize for the student or postdoc whose work had the highest potential impact on the understanding of human cancer.

F. Morales, J. Molina, M. Moral

L. Trotman, P. Paolo Pandolfi, F. Furnari

PROGRAM

Signaling Upstream of PIP3

Signaling Downstream from PIP3

Therapeutic Targets in PI3K/PTEN Signaling

PTEN Pathways and Disease

PTEN Pathways and Cancer

PTEN Regulation and PTEN/PI3K Pathway Cross-talk

A. Ross

I. Berenjeno, J. Guillermet-Guibert

N. Leslie, B. Eickholt

Neuronal Circuits: From Structure to Function

March 13–16 176 participants

ARRANGED BY **Ed Callaway,** Salk Institute for Biological Sciences
Dmitri Chklovskii, Cold Spring Harbor Laboratory
Liqun Luo, Stanford University

In the quest to understand the brain, neuronal circuits represent a central level of description. Establishing connectivity in neuronal circuits seems to be as essential for solving the brain as having a geographic map for planning one's travel. Although such realization has motivated Santiago Ramón y Cajal to describe a variety of neuronal circuits using Golgi stains, his contributions were technologically limited. With the recent appearance of novel molecular genetic, imaging, and computational techniques, a comprehensive description of the wiring diagram—an old dream of neuroscientists—is about to become a reality.

E. Callaway, L. Luo, D. Chklovskii

Because technological advances have been made in different organisms and systems, we wanted to create a forum that brings together researchers working on different topics, yet focusing on neuronal circuits. This meeting included six broad slide sessions covering the olfactory system, the visual system, methods, behavior learning and memory, motor systems and the cortex, and a very interactive poster session, including an opening mix followed by presentations on Chemical senses, Visual system, Circuits underlying cortical function, Motor control, and New methods for investigating structure and function of circuits.

For the second meeting of this kind, the response from the field was very enthusiastic. The meeting brought together participants from all over the world, most of whom made either oral or poster presentations. Invited talks were given by Cori Bargmann, The Rockefeller University; Tobias Bonhoeffer, Max-Planck Institute of Neurobiology; Karl Deisseroth, Stanford University; Winfried Denk, Max-Planck Institute for Medical Research; Jeff Lichtman, Harvard University; Eve Marder, Brandeis University; Edvard Moser, Center for the Biology of Memory; Sacha Nelson, Brandeis University; Clay Reid, Harvard Medical School; Massimo Scanziani, University of California, San Diego; Kristin Scott, University of California, Berkeley; Stephen Smith, Stanford Medical School; Charles Stevens, The Salk Institute; Karel Svoboda, Janelia Farm Research Campus, HHMI; Gabor Tamas, University of Szeged, Hungary/HHMI; Rachel Wilson, Harvard Medical School; and Rafael Yuste, Columbia University/HHMI. 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, network, and 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.

A highlight of the meeting program was the inaugural Larry Katz Memorial Lecture, thanks to the generous donation of many colleagues in the field; 25 excellent nominations were received from all over the world. A committee composed of the three organizers and Cori Bargmann selected two speakers: Andrea Hasenstaub of the Salk Institute for Biological Studies and Feng Zhang of Stanford University. Both gave wonderful talks about their graduate studies.

C. Reid, T. Bonhoeffer, D. Bock

PROGRAM

Opening Mix
D. Chklovskii, *HHMI/Janelia Farm Research Campus, Ashburn, Virginia*

Cortex
E.M. Callaway, *Salk Institute for Biological Studies, La Jolla, California*
Chairperson: L. Luo, *Stanford University, California*

Larry Katz Memorial Lectures
Introduction and Award Presentation: D. Katz and R. Yuste
A. Hasenstaub, *Salk Institute for Biological Studies*
F. Zhang, *Stanford University, California*

Molecular Genetics Approaches
Chairperson: L. Luo, *Stanford University, California*

Chemical and Other Senses
Chairperson: L. Luo, *Stanford University, California*

Visual System/Neurogenesis
Chairperson: D. Chklovskii, *HHMI/Janelia Farm Research Campus, Ashburn, Virginia*

Methods
Chairperson: E.M. Callaway, *Salk Institute for Biological Studies, La Jolla, California*

S. Kuhlman, X. Wu

W. Grimes, O. Ahmed

K. Scott, M.L. Vasconcelos

Y. Kozorovitskiy

W. Denk, D. Chklovskii

Epithelial–Mesenchymal Transition

March 17–20 161 Participants

ARRANGED BY **Raghu Kalluri,** Harvard Medical School
John D. Haley, OSI Pharmaceuticals
Senthil Muthuswamy, Cold Spring Harbor Laboratory

Epithelial–mesenchymal transition (EMT) is a biological program that induces a change in cell fate characterized by a loss in the epithelial phenotype and acquisition of a mesenchymal state. EMT is essential for several developmental processes including mesoderm formation and neural tube formation and appears to be critical for fibrosis and cancer cell progression associated with invasion and metastasis. EMT and the accompanying increase in cell mobility is associated with loss of expression of epithelial keratins and E-cadherins and an increase in expression of mesenchymal keratins and integrins. Several signal transduction pathways, such as TGF-β, Ras-MAPK, PI3K-Akt, and Wnt, and transcription factors of the Snail family, have been shown to be involved in regulation of EMT.

S. Muthuswamy, J. Haley, R. Kalluri

This new meeting addressed recent advances in the understanding of the cell biological and molecular mechanisms that regulate EMT. Topics covered by the meeting included but were not limited to biology of EMT in development, inflammation, and fibrosis; extracellular signaling and cell biology of EMT; intracellular regulators of EMT; EMT models and stem cells; and pathobiology of EMT in cancer progression. The meeting included two keynote addresses by Mina Bissell (Lawrence Berkeley National Laboratory) and Robert Weinberg (Whitehead Institute for Biomedical Research).

The meeting attracted 161 participants from more than ten countries. Oral presentations were supplemented by two afternoon poster session that were extremely well attended and provided a forum for all meeting participants, including many graduate students and postdocs, to share and discuss their most recent data.

The meeting was supported by a generous grant from OSI Pharmaceuticals.

PROGRAM

Keynote Address 1: EMT and the Stem Cell State
Robert Weinberg, *Whitehead Institute for Biomedical Research*

Keynote Address 2: Architecture and Shape Are Involved in Matrix Metalloproteinase-induced EMT
Mina Bissell, *Lawrence Berkeley National Laboratory*

Biology of EMT in Development, Inflammation, and Fibrosis
Chairpersons: R. Kalluri, *Harvard Medical School, Boston, Massachusetts;* E. Williams, *Monash Institute of Medical Research, Clayton, Australia*

Extracellular Signaling and Cell Biology
Chairpersons: J. Rosen, *Baylor College of Medicine, Houston, Texas;* S. Muthuswamy, *Cold Spring Harbor Laboratory*

M. Bissell

Intracellular Regulators of EMT
Chairpersons: F. Rauscher, *Wistar Institute, Philadelphia, Pennsylvania;* A. Nieto, *Instituto de Neurociencias Alicante CSIC-UMH, Spain*

EMT Models and Stem Cells
Chairpersons: E.W. Thompson, *St. Vincent's Institute of Medical Research, Fitzroy, Australia;* J. Condeelis, *Albert Einstein College of Medicine, Bronx, New York*

Pathobiology of EMT in Cancer Progression
Chairpersons: J.P. Thiery, *Institute of Molecular and Cell Biology, Singapore;* S. Dubinett, *David Geffen School of Medicine, Los Angeles, California*

S. Kowli

T. Grodzicker, D. Stewart, J. Rosen, M. Bissell

R. Weinberg, G. Longmore

J. Woodgett, J. Wrana

S. Barr

CSHL/WELLCOME TRUST CONFERENCE

These conferences were held at the Hinxton Hall Conference Centre, located several miles south of Cambridge in the United Kingdom, which forms part of the Wellcome Trust Genome Campus, together with the Sanger Institute and the European Bioinformatics Institute. The conferences are managed jointly by Cold Spring Harbor Laboratory and the Wellcome Trust and follow Cold Spring Harbor style in that the majority of talks are chosen from openly submitted abstracts. The topics of the joint conference series emphasize genomics and bioinformatics, or topics of particular interest to science in the United Kingdom or Europe.

Computational Cell Biology

March 26–29 69 participants

ARRANGED BY **Benjamin Geiger,** Weizmann Institute
Edda Klipp, Max-Planck Institute for Molecular Genetics
Bela Novak, Oxford Centre for Integrative Systems Biology

Following the success of the first Cold Spring Harbor Laboratory meeting on Computational Cell Biology in March 2007, a second conference on the topic was held at the Wellcome Trust Conference Centre on the Wellcome Trust Genome Campus in Hinxton, United Kingdom. The specific goal for this meeting was to foster fruitful and creative dialogs between experimental cell biologists and mathematical—computational modelers with common interests in the regulation of cell physiology.

PROGRAM

Software Session
Chairperson: P. Mendes, *University of Manchester, United Kingdom*

Keynote Address: Self-organization
E. Karsenti, *European Molecular Biology Laboratory, Heidelberg, Germany*

Growth and Division
Chairperson: B. Novak, *Oxford Centre for Integrative Systems Biology, United Kingdom*

Motility and Cytoskeleton
Chairperson: B. Geiger, *Weizmann Institute of Science, Rehovot, Israel*

Keynote Address: What does a living cell know of itself?
D. Bray, *University of Cambridge, United Kingdom*

Microbial Physiology
Chairperson: J. Armitage, *University of Oxford, United Kingdom*

Signaling
Chairperson: E. Klipp, *Max-Planck Institute for Molecular Genetics, Berlin, Germany*

Software Demonstrations

Circadian Clock
Chairperson: H. Ueda, *RIKEN Center for Developmental Biology, Kobe, Japan*

Development
Chairperson: A. Oates, *Max-Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany*

Aerial view over the Francis Crick Auditorium and the Genome Campus. The Sanger Institute is visible in the background.

Network Biology

August 27–31 88 participants

ARRANGED BY **Anne-Claude Gavin,** European Molecular Biology Laboratory
Trey Ideker, University of California, San Diego
Marc Vidal, Dana-Farber Cancer Institute/Harvard
Marian Walhout, University of Massachusetts Medical School

This fourth conference on Network Biology was held at the Wellcome Trust Genome Campus in Hinxton, United Kingdom and attracted an audience of senior and junior investigators and postdoctoral and (post)graduate researchers in a range of disciplines who sought to share existing research and experience. The conference addressed topics including ORFeome and other clone resources; Y2H and other binary assay maps; pull-down mass spectrometry approaches; orthogonal binary assays; assembly/annotation; integration with phenotypic, transcriptome, and localization clustering data; domain–domain networks; and interaction-defective genetics.

PROGRAM

Transcriptional and Posttranscriptional Gene Regulatory Networks
Chairperson: R. Albert, *Pennsylvania State University, University Park, Pennsylvania*

Keynote Address: The evolution and function of domain-based protein interaction networks
T. Pawson, *Samuel Lunenfeld Research Institute, Toronto, Canada*

Signaling and Machinery Networks
Chairperson: E. Furlong, *European Molecular Biology Laboratory, Heidelberg, Germany*

Metabolic Networks
Chairperson: E. Schadt, *Rosetta Inpharmatics, Merck & Co., Seattle, Washington*

From Network to Protein Properties
Chairperson: A.-L. Barabasi, *Northeastern University, Boston, Massachusetts*

Genetic Networks
Chairperson: B. Palsson, *University of California, San Diego*

Network Medicine
Chairperson: B. Luisi, *MRC Institute of Virology, Glasgow, United Kingdom*

Genomic Perspectives to Host-pathogen Interactions

September 3–6 73 participants

ARRANGED BY **Matthew Berriman,** Wellcome Trust Sanger Institute
Julian Parkhill, Wellcome Trust Sanger Institute
George Weinstock, Washington University School of Medicine

This third conference covered free-living, facultative, opportunistic, epizootic, obligate, and intracellular pathogens; lung, gastrointestinal, urogenital, skin, muscle, central nervous system, blood, and lymph host niche/defense; and genome, biological, and ecological vectors. 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 Center.

PROGRAM

Microbial Evolution
Chairpersons: R. Hirt, *Newcastle University, United Kingdom;* D. Conway, *Medical Research Council Laboratories, The Gambia*; M. Maiden, *University of Oxford, United Kingdom*

Metagenomics
Chairpersons: O.C. Stine, *University of Maryland School of Medicine, Baltimore;* G. Weinstock, *Washington University School of Medicine, St. Louis, Missouri*

Virus Discovery
Chairpersons: E. Holmes, *Pennsylvania State University, University Park, Pennsylvania;* E. Ghedin, *University of Pittsburgh, Pennsylvania*

Vectors/Host
Chairpersons: D. Masiga, *International Centre of Insect Physiology and Ecology, Nairobi, Kenya;* C. Hill, *Purdue University, West Lafayette, Indiana*

Parasites
Chairpersons: J. Gilleard, *University of Glasgow, United Kingdom;* W. Petri, *University of Virginia, Charlottesville*

Bacteria
Chairpersons: M. So, *University of Arizona School of Medicine, Tucson;* N. Thomson, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* G. Pluschke, *Swiss Tropical Institute, Basel, Switzerland*

Keynote Address
J. Farrar, *Oxford University Clinical Research Unit, The Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam*

Genome Informatics

September 10–14 169 participants

ARRANGED BY **Michele Clamp,** The Broad Institute of MIT and Harvard
James Kent, University of California, Santa Cruz
Jason Swedlow, University of Dundee

This eighth conference was held at the Wellcome Trust Conference Centre on the Wellcome Trust Genome Campus in Hinxton, United Kingdom and focused on large-scale genome informatics. Biology is an experimental science that is experiencing an explosion of new data. This requires biologists to increase the scale and sophistication of the information technology used for their research. The conference scope encompassed the management and analysis of these data, such as whole-genome comparisons within and among species and strains, analysis of results from high-throughput experiments to uncover cellular pathways and molecular interactions, and the design of effective algorithms to identify regulatory sequence motifs.

PROGRAM

Pathogens and Medical Genomics
Chairpersons: P. Taschner, *Leiden University Medical Center, The Netherlands;* J. Galagan, *The Broad Institute of MIT and Harvard, Cambridge, Massachusetts*

Assembly and Informatics for New Sequencing Technologies
Chairperson: R. Durbin, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Epigenomics and Gene Regulation
Chairpersons: B. Ren, *Ludwig Institute for Cancer Research, La Jolla, California;* M. Eisen, *University of California, Berkeley*

Images, Atlases, and Reconstruction
Chairperson: M. Hawrylycz, *Allen Institute for Brain Science, Seattle, Washington*

Keynote Address
J. Thornton, *EMBL Outstation, Hinxton, United Kingdom*

Data Management, Mining, Curation, and Visualization
Chairperson: J. Taylor, *New York University, New York*

Comparative and Evolutionary Genomics
Chairperson: A. Sidow, *Stanford University School of Medicine, California*

Protein Informatics: Evolution, Interactions, and Functional Prediction
Chairpersons: T. Hubbard, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* M. Vidal, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Integrative Approaches to Brain Complexity

October 1–5 55 participants

ARRANGED BY **Seth Grant,** Wellcome Trust Sanger Institute
Nathaniel Heintz, HHMI/The Rockefeller University
Jeffrey Noebels, Baylor College of Medicine

This fifth conference addressed approaches ranging from molecular biology to behaving animal studies, from single genes to complex sets of genes, from synapses to networked brain functions. Emphasis was placed on large-scale approaches that generate accessible molecular and biological databases. 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 Center.

PROGRAM

Welcome and Outline of Meeting
S. Grant, *Wellcome Trust Sanger Institute, United Kingdom*
N. Heintz, *HHMI/The Rockefeller University, New York*
J. Noebels, *Baylor College of Medicine, Houston, Texas*

Neurotransmitter Receptors
Chairperson: P. Seeburg, *University of Heidelberg, Germany*

Synaptic Computation
Chairperson: A. Silver, *University College London, United Kingdom*

Dendrites
Chairperson: E. Schuman, *HHMI/California Institute of Technology, Pasadena*

Modeling Neuronal Networks
Chairperson: S. Grillner, *Karolinska Instituet, Stockholm, Sweden*

Francis Crick Lecture in Neuroscience
Chairperson: N. Unwin, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

Neural Networks
Chairperson: K. Deisseroth, *Stanford University, California*

Inhibitory Networks
Chairperson: H. Monyer, *University of Heidelberg, Germany*

Networks in Disease I and II
Chairperson: J. Noebels, *Baylor College of Medicine, Houston, Texas*

Emerging Technologies
Chairperson: N. Heintz, *HHMI/The Rockefeller University, New York*

Behavior
Chairperson: J. LeDoux, *New York University, New York*

Rat Genomics and Models

December 3–6 104 participants

ARRANGED BY **Edwin Cuppen,** Hubrecht Institute, The Netherlands
Norbert Hübner, Max-Delbruck Center, Germany
Anne Kwitek, University of Iowa, Ames
James Shull, University of Nebraska Medical Center

Building upon the strength of previous biennial CSHL meetings held since 1999, this first Joint Cold Spring Harbor/Wellcome Trust conference took place in December. The meeting addressed the uses of the rat in genetics, genomics, and disease models. The Wellcome Trust Genome Campus is situated south of Cambridge and north of Stansted Airport, within easy reach of either destination. Attendees to the conference were treated to unusually mild weather.

PROGRAM

Genome Biology
Chairpersons: S. Mockrin, *NHLBI, National Institutes of Health, Bethesda, Maryland;* G.M. Lathrop, *Centre National De Genotypage, Evry, France*

Keynote Address
A. Bradley, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Cardiovascular/Renal Disease
Chairpersons: S. Old, *NHLBI, National Institutes of Health, Bethesda, Maryland;* M. Pravenec, *Czech Academy of Sciences, Prague*

Metabolism/Inflammation
Chairpersons: T. Aitman, *Imperial College, London, United Kingdom;* D. Gauguier, *Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom*

Tumor Biology
Chairpersons: W. Dove, *University of Wisconsin, Madison;* J. Shull, *University of Nebraska Medical Center, Omaha*

Neurosciences
Chairpersons: R. Murphy, *University College London, United Kingdom;* P. Carninci, *RIKEN, Yokohama, Japan*

Genomic Resources
Chairpersons: X. Fernandez, *European Bioinformatics Institute, Hinxton, United Kingdom;* M. Shimoyama, *Medical College of Wisconsin, Milwaukee*

Emerging Technologies and Translation to Humans
Chairperson: D. Schwartz, *University of Wisconsin, Madison*

Hinxton Hall

Conference Center and Hall, side view

Wellcome Trust Conference Center, Main Conference Center and Hinxton Hall

Systems Biology: Global Regulation of Gene Expression

March 27–30 289 participants

ARRANGED BY **Julia Bailey-Serres,** University of California, Riverside
Martha Bulyk, Brigham and Womens Hospital and Harvard Medical School
Harmen Bussemaker, Columbia University
Bing Ren, University of California, San Diego/Ludwig Institute for Cancer Research

Systems biology aims to understand the emergent properties of molecular networks in cells by using systematic and global approaches. One of the most actively researched areas of systems biology in recent years has been global regulation of gene expression, which coordinates complex metabolic and developmental programs in organisms. This sixth conference, like the ones in previous years, once again captured the rapid progress and many new discoveries in this young and exciting field. A total of 289 scientists attended the 3-day meeting, which featured 46 talks and 109 poster presentations covering a broad range of topics. Dr. Robert Waterston, a pioneer in genome sciences, gave a keynote speech describing the *C. elegans* modENCODE project that uses an integrated approach to discern the gene regulatory networks controlling embryogenesis of this model organism. Following this presentation was seven oral sessions devoted to specific themes, including epigenetics, genomic regulatory sequences, gene regulatory networks, noncoding RNAs, proteomics, and emerging technologies. Compared to previous conferences, this year's meeting featured prominently in four aspects: (1) Next-generation sequencing technologies have been a key technological component in a great number of projects, and these have stimulated the generation of a plethora of new genomic methods, computational algorithms, resources, and biological insights; (2) new imaging technologies are allowing researchers to examine the dynamics of gene expression in vivo and in real time and, in some cases, at a single-molecule level; (3) new resources on transcription-factor-binding specificities have been generated, providing opportunities for systematic analysis of gene regulatory networks in *Drosophila* and mammals; and (4) new computational algorithms have been adopted to analyze the gene regulatory networks. Additionally, the speakers in these sessions were generally young, with most being in the early stage of their careers. Like in the previous year, this year's conference also included two poster presentation sessions, and the *Genome Research* best-poster prize was awarded to Rickard Sandberg (from the Karolinska Institute) for "Widespread evasion of posttranscriptional regulation associated with proliferation." Finally, to help computational biologists and experimental scientists to better communicate with one another, this year's meeting also included a premeeting workshop that focused on introductory experimental and computational topics.

I. Ladunga, R. Waterston

This meeting was funded, in part, by the National Science Foundation.

T. Degenhardt, A. Boorsma, M. Radonjic

PROGRAM

Keynote Address
R. Waterston, *University of Washington, Seattle*

Epigenetics
Chairperson: B. Ren, *Ludwig Institute for Cancer Research, La Jolla, California*

Genomic Regulatory Elements I
Chairperson: H. Bussemaker, *Columbia University, New York*

Genomic Regulatory Elements II
Chairperson: H. Bussemaker, *Columbia University, New York*

Posttranscriptional Regulation
Chairperson: J. Bailey-Serres, *University of California, Riverside*

Transcriptional Regulatory Networks
Chairperson: M. Zhang, *Cold Spring Harbor Laboratory*

Emerging Technology
Chairperson: T. Hughes, *University of Toronto, Canada*

Proteomics
Chairperson: M. Bulyk, *Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts*

B. Ren, C. Zhang

M. Bulyk, J. Bailey-Serres, H. Bussemaker

V. Kiermer, T. Chouard

D. Ware, H. Sussman

N. Friedman, D. Gifford

iPlant Collaborative: Grand Challenges in Plant Sciences

April 7–9 196 participants

ARRANGED BY **The iPlant Collaborative**

This adjunct meeting was sponsored by The *iPlant* Collaborative (iPC), a major new National Science Foundation–funded initiative in cyberinfrastructure for plant sciences. The iPC is intended to be fluid and dynamic, using new computer, computational science, and cyberinfrastructure solutions to address an evolving array of grand challenges in the plant sciences. It aims to be community-driven, involving plant biologists, computer and information scientists, and engineers, as well as experts from other disciplines, all working in integrated teams. The iPC brings together strengths in plant biology, bioinformatics, computer science, and high-throughput computing as well as innovative approaches to education and outreach and the study of social networks. This inaugural meeting was intended to promote discussion of a wide range of topics and to begin the process of building a community-owned, distributed collaborative addressing compelling "grand challenges" for the plant and computing sciences including (1) From molecules to whole organisms: Pathways, systems, and networks in metabolism, physiology, development, and organismic biology; (2) From molecules to ecosystems: Biochemistry, genetics, population biology, and ecology; and (3) The evolutionary diversification of function: From molecular, cellular, and developmental biology to comparative genomics and phylogenetics. The meeting was intended to be highly interactive, and parallel sessions were enthusiastically attend-

David Stewart monitoring three simultaneous live web streams of the conference

Interactive break-out session in Bush Auditorium

ed and simultaneously streamed over the internet to groups at remotes sites around the country. These sessions allowed for energetic discussion about the development of Discovery Environments or prototype cyberinfrastructures for discovery, collaboration, and computational thinking in biology. Throughout the meeting, social events provided ample opportunity for informal interactions and discussions of further collaborative efforts.

This meeting was funded, in part, by the National Science Foundation.

S. Ram, S. Smith

Panel discussion

D. Jackson, J.-P. Vielle-Calzada

Gene Expression and Signaling in the Immune System

April 22–26 472 participants

ARRANGED BY **Doreen Cantrell,** University of Dundee
Richard Flavell, HHMI/Yale University, New Haven
Mark Schlissel, University of California, Berkeley
Stephen Smale, University of California School of Medicine, Los Angeles

This meeting was held for the fourth time in 2008, basking in the bright sunshine and mild temperatures of springtime. More than 470 registrants (the largest number ever), including about 40% from outside of the United States and 66% first-time attendees, participated in a highly engaging and interactive 4-day meeting. Although there are many immunology meetings, the unique aspect of this Cold Spring Harbor meeting is its focus on molecular and biochemical aspects of the development and function of the immune system. In addition, this meeting attracts a broad range of scientists who use the immune system as a model to ferret out basic principles of biological regulation. Support was obtained from the NIH/NIAID as well as from seven corporate sponsors. Talks were presented by a mix of invited speakers and investigators selected from among a group of more than 200 submitted abstracts. A significant number of women and junior investigators were asked to give talks. Most speakers focused almost exclusively on their unpublished work. A recurrent theme and scientific highlight of the meeting was the role of chromatin structure as a target of signaling pathways in the regulation of gene expression. Keji Zhao (National Institutes of Health) presented the results of a comprehensive genome-wide study of posttranslational histone modification (20 different covalent marks in addition to the localization of RNA pol II) in T lymphocytes that is contributing to a surprising new conceptual framework for gene regulation. Marjorie Oettinger (MGH/Harvard Med) presented work showing that RAG2 (an essential component of the lymphoid V[D]J recombinase) binds specifically with K4-trimethylated histone H3 and that this interaction is essential for normal recombinase activity in vivo. Cornelius (Kees) Murre (University of California, San Diego) presented the first three-dimensional model of interphase structure of a complex genetic locus within nuclear space. The product of a collaboration with physicists and mathematicians, this model based on flu-

S. Smale, S. Chan

D. Kioussis, M.A. Vidal

S. Szabo, K. Mowen

orescent in situ hybridization data helps to explain how more than 100 immunoglobulin V_H gene segments distributed over greater than a megabase of DNA can recombine with D_H segments at roughly similar frequencies. Finally, in the last talk of the meeting, Ruslan Medzhitov (Yale) presented data indicating that genes responding most rapidly to inflammatory stimuli are "preloaded" with RNA polymerase bound to their promoters and that the signal to turn on transcription consists of precise alterations in local histone modifications and cofactor activity in response to transcription factor activation. These talks highlight the relevance of the data discussed at this meeting not just to immunology, but to the broader scientific community. Oral presentations were supplemented by three afternoon poster sessions that were extremely well attended and provided a forum for all meeting participants (including many graduate students and postdocs) to share their most recent data. In addition, attendees took advantage of walking tours of the CSHL campus and an evening performance by the Yale-based music group "The Cellmates."

K. Rajewsky, A. Tarakhovsky

This meeting was funded, in part, by Abbott Bioresearch, Amgen, ChemoCentryx, Elan Pharmaceuticals, Eli Lilly and Company, Genetech, IRX Therapeutics, R & D Systems, Vertex Pharmaceuticals, and the National Institute of Allergy and Infectious Disease, a branch of the National Institutes of Health.

PROGRAM

Stem Cells and Early Developmental Decisions
Chairperson: C. Guidos, *Hospital for Sick Children, Toronto, Canada*

Regulation of Immune Cell Development
Chairperson: D. Baltimore, *California Institute of Technology, Pasadena*

Chromatin Structure and Epigenetic Regulation
Chairperson: R. Sen, *NIA/National Institutes of Health, Baltimore, Maryland*

Antigen Receptor Gene Assembly and Modification
Chairperson: M. Schlissel, *University of California, Berkeley*

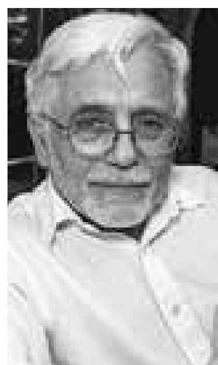
Signal Transduction in Immune Cells
Chairperson: D. Cantrell, *University of Dundee, United Kingdom*

Regulation of Lymphocyte Function I
Chairperson: R. Flavell, *Yale University School of Medicine, New Haven, Connecticut*

Regulation of Lymphocyte Function II
Chairperson: D. Raulet, *University of California, Berkeley*

Innate Immunity
Chairperson: S. Smale, *University of California School of Medicine, Los Angeles*

R. Flavell, R. Medzhitov


P. Kisielow

T. Mak, S. Chen-Kiang

Molecular Chaperones and Stress Responses

April 30–May 4 299 participants

ARRANGED BY **James Bardwell,** HHMI/University of Michigan
F. Ulrich Hartl, Max-Planck Institute for Biochemistry
David Ron, New York University School of Medicine

This meeting continued the rich tradition of a forum where cutting-edge analysis of molecular mechanisms of protein folding is presented in the context of its biological importance and relevance to health.

D. Ron, A. Rousaki

New structural insights into the mechanisms of chaperone action were provided, with, for the first time, two crystallographic views of an entire DnaK/Hsp70 molecule, shedding light on the coupling of nucleotide binding and hydrolysis to substrate binding in this highly conserved folding machine. Significant advances were also reported in the understanding of the related Hsp90 chaperone, this time using solution methods. New insights were provided into the coupling of translation with protein folding in studies that point to the role of ribosome as both the site of mRNA translation and the earliest node in a series of folding machines. These presentations represent an interesting evolution in conference, attracting leaders in ribosome biology and crystallography and students of the membrane translocon. Innovations in the time-resolved study of chaperone-assisted protein folding at the single-molecule level were described, and this shed light on the action of chaperonins.

New insights into the cellular context of chaperone action in protein import into organelles and in retrotranslocation of misfolded proteins from the ER were described; the latter included the first description of a PDI chaperone dedicated to the process of ER-associated protein degradation and talks on the role of lectins in recognizing cellular tags that mark proteins for destruction. The itinerary of misfolded cytoplasmic proteins was described in elegant studies in yeast, integrating cell biology with more classic chaperone biochemistry. The latter emerged as a theme at this meeting that now encompasses a broad community of workers in diverse disciplines.

The session devoted to diseases of misfolded proteins featured talks on interactions of prions and misfolded poly-Q-expanded proteins with molecular chaperones, with new insight into the function of

J. Tatzelt, L. Bouman, J. Schlehe

K. Winkelhofer, R. Morimoto

deaggrating machines. The study of signaling pathways responsive to protein misfolding and unfolded protein stress has been among the core interests of the meeting's attendees, but over time, this has evolved to encompass not only the cytosolic heat shock response, whose involvement in cancer received coverage by several presentations, but also stress signaling from organelles. In the latter realm, the first crystallographic study of the signaling portion of IRE1 involved in the ER stress response was presented.

The session on protein degradation brought new insights into the molecular mechanism of action of the simpler bacterial ClpXP and new insights into the composition and function of the more complicated proteasome.

PROGRAM

Cellular Response to Stress
Chairperson: R. Morimoto, *Northwestern University, Evanston, Illinois*

Chaperone Biochemistry and Protein Folding
Chairperson: J. Buchner, *Technische Universität München, Germany*

Chaperone Function in Disease and Development
Chairperson: L. Neckers, *NCI/National Institutes of Health, Bethesda, Maryland*

Diseases of Protein Misfolding
Chairperson: D. Selkoe, *Harvard Medical School, Boston, Massachusetts*

Quality Control and Protein Trafficking
Chairperson: A. Johnson, *Texas A&M University Health Science Center, College Station*

Regulation of the Stress Response
Chairperson: C. Gross, *University of California, San Francisco*

Chaperones and Proteolysis
Chairperson: D. Finley, *Harvard Medical School, Boston, Massachusetts*

Poster session

O. Lipan

C. Ruiz

H. Furukawa, W. Hendrickson

The Biology of Genomes

May 6–10 553 participants

ARRANGED BY **Michael Ashburner,** University of Cambridge
Andrew Clark, Cornell University
Kerstin Lindblad-Toh, Broad Institute
George Weinstock, Washington University

This meeting marked the 20th annual gathering of genome scientists in this setting. The past decade or so has seen remarkable progress not only in genome sequencing, but also in the application of sequencing technologies to a wide range of biomedical problems. The first two talks of this meeting announced two new individual human sequences, and subsequently we heard about the sequencing of genomes of organisms as different as the platypus and the parasitic wasp. There was considerable discussion about the newly funded project to sequence the genomes of 1000 individual humans.

O. Bahcall, M. Ashburner

Just over 550 people from around the world attended the meeting, with more than 370 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms. This was, in fact, a record attendance for a Cold Spring Harbor meeting, and, unfortunately, well over 100 people who wanted to attend, could not for reasons of space.

The session topics included areas such as biological insights from genetics and genomics of non-human species, high-throughput genetics and genomics, cancer and functional genomics, computational genomics, population genomic variation, genetics of complex traits, and evolutionary genomics. There was considerable enthusiasm for the new resequencing technologies that are becoming available and the science that these technologies will enable. Indeed, it could be said that information acquired by means of these technologies dominated the meeting, and all had the very real sense of a major advance having been made in the field.

J. Watson

The keynote talks were chosen to complement the major sessions of the meeting. They were presented by Dr. Michael Levine (University of California, Berkeley) and Dr. Michael Lynch (Indiana University, Bloomington).

The ELSI (Ethical, Legal, and Social Implications) panel chaired by Francis Collins focused on ethical and social implications of research on natural selection in humans. This year's ELSI session was particularly well attended and was addressed by senior figures in the emerging field of commercial human genome sequencing: Dr. Kari Steffansson (deCODE), Linda Avey (23andMe), and Dietrich Stephan (Navigenics), with academic contributions from Kathy Hudson (Genetics and Public Policy Center) and Joseph McInerney (National Coalition for Health Professional Education in Genetics).

This meeting was funded, in part, by the National Human Genome Research Institute, a branch of the National Institutes of Health, and Roche Applied Science. Interviews were supported by the HudsonAlpha Institute for Biotechnology.

PROGRAM

Functional and Cancer Genomics
Chairpersons: R. Wilson, *Washington University, St. Louis, Missouri;* M. Dean, *National Cancer Institute, Frederick, Maryland*

Genetics of Complex Traits
Chairpersons: M. Georges, *University of Liège, Belgium;* A. DiRienzo, *University of Chicago, Illinois*

High-throughput Genomics and Genetics
Chairpersons: M. Vidal, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts;* S. Celniker, *Lawrence Berkeley National Laboratory, California*

Computational Genomics
Chairpersons: S. Salzberg, *University of Maryland, College Park;* S. Lewis, *Lawrence Berkeley National Laboratory, California*

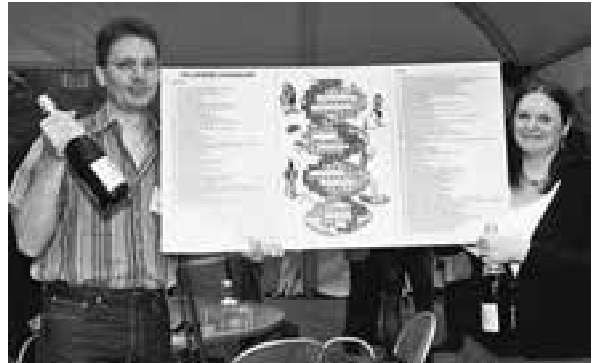
ELSI Panel Discussion: Ethical, Legal, And Social Implications of Direct-to-Consumer Genomic Test Marketing
Moderator: Francis Collins, M.D., Ph.D., *National Human Genome Research Institute*
Panelists: Kari Stefansson, Ph.D., *deCODEme;* Dietrich Stephan, Ph.D., *Navigenics;* Linda Avey, *23andMe;* Kathy Hudson, Ph.D., *Genetics and Public Policy Center;* Joseph McInerney, M.S., *National Coalition for Health Professional Education in Genetics (NCHPEG)*

Evolutionary Genomics
Chairpersons: J. Noonan, *Yale University, New Haven, Connecticut;* S.L. Baldauf, *University of York, United Kingdom*

Genetics and Genomics of Nonhuman Species
Chairpersons: G. Barsh, *Stanford University, California;* W. Warren, *Washington University School of Medicine, St. Louis, Missouri*

Guest Speakers
Michael Levine, *University of California, Berkeley;* Michael Lynch, *Indiana University, Bloomington*

Population Genomic Variation
Chairpersons: E. Eichler, *University of Washington, Seattle;* M. Przeworski, *University of Chicago, Illinois*



T. Taylor, E. Bruford

D. Schwartz, D. Ware

H. Sussman, T. Hubbard

Watching the meeting under the Grace tent

The Cell Cycle

May 14–18 314 participants

ARRANGED BY **Orna Cohen-Fix,** NIDDK, National Institutes of Health
Nicholas Dyson, Massachusetts General Hospital Cancer Center
Johannes Walter, Harvard Medical School

N. Dyson, O. Cohen-Fix, J. Walter

This ninth biannual Cell Cycle meeting was held this year at Cold Spring Harbor. This conference is internationally recognized for its ability to bring together scientists who study cell cycle regulation in eukaryotes ranging from yeast to humans. As usual, the meeting began with a keynote talk from a leader in the field, and on this occasion, we were fortunate to have Dr. Timothy Mitchison (Department of Systems Biology at Harvard Medical School) tell us about his exciting new studies of spindle structure and dynamics. The remainder of the meeting was organized around eight lecture sessions and two poster sessions that focused on the major stages of the cell division cycle, with an emphasis in each session on the molecular mechanisms that govern cell cycle progression. Many of these sessions focused on long-standing problems in cell cycle control, including the transcriptional mechanisms controlling entry in the cell cycle, the mechanisms that initiate chromosome duplication and limit it to a single round, the mechanisms that trigger the complex events of chromosome segregation in mitosis, and the numerous checkpoint systems that ensure that cell cycle events occur in the correct order. For the first time, an entire session was devoted to the involvement of proteolysis in cell cycle regulation, bringing together different perspectives from investigators who study the role of proteolysis in a variety of cell cycle processes. Other emerging areas, including molecular insights into the regulation of endoreduplication and the tensile properties of the kinetochore, were also represented. As always, every major model system for cell cycle analysis, including plants, was represented, and the striking phylogenetic conservation of cell cycle regulatory mechanisms was readily evident. The 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 progress in this field. It was another landmark meeting for the cell cycle field, and the participants continue to look forward to equally exciting meetings in future years.

This meeting was funded, in part, by the National Cancer Institute, a branch of the National Institutes of Health.

Unusual place for a poster!

C. Murphy

PROGRAM

Cell Cycle Transitions
Chairperson: D. Morgan, *University of California, San Francisco*

Keynote Address: Spindle Structure and Dynamics
T. Mitchison, *Harvard Medical School*

G_1 and the G_1/S Transition
Chairpersons: C. Wittenberg, *Scripps Research Institute, La Jolla, California;* P. Sicinski, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

DNA Replication
Chairpersons: H. Araki, *National Institute of Genetics, Mishima, Japan;* P. Russell, *Scripps Research Institute, La Jolla, California*

The Response to DNA Damage and Perturbation in DNA Replication
Chairpersons: M. Michael, *Harvard University, Cambridge, Massachusetts;* H. Ulrich, *Cancer Research UK London Research Institute*

Cell Cycle Regulatory Pathways
Chairpersons: J. Kimble, *HHMI/University of Wisconsin, Madison;* S. Kornbluth, *Duke University Medical Center, Durham, North Carolina*

Mechanisms and Regulation of Chromosome Segregation
Chairpersons: S. Biggins, *Fred Hutchinson Cancer Research Center, Seattle, Washington;* K. Labib, *Cancer Research UK/Paterson Institute for Cancer Research, Manchester*

Metaphase, Anaphase, and Beyond
Chairpersons: K. Gould, *HHMI/Vanderbilt University, Nashville, Tennessee;* T. Orr-Weaver, *Whitehead Institute/Massachusetts Institute of Technology, Cambridge, Massachusetts*

Proteolysis in Cell Cycle Regulation
Chairpersons: P. Jackson, *Stanford University School of Medicine, California;* R. King, *Harvard Medical School, Boston, Massachusetts*

D. Morgan, M. Solomon

A. Lasada, I. Sumara

M. De Pamphilis, J. Mendez

Retroviruses

May 19–24 475 participants

ARRANGED BY **Paul Spearman,** Emory University
Alice Telesnitsky, University of Michigan

This conference focused on recent developments in basic retrovirology, including HIV, HTLV-I, and the broad array of nonhuman retroviruses. Keynote speakers were Jan Svoboda of the Academy of Sciences of the Czech Republic and John Wills of Penn State College of Medicine. Dr. Svoboda is one of the pioneers of retrovirology. During the early years of RNA tumor virology, he described the unusual phenomenon of Rous sarcoma virus rescue from rat XC cells through coculture with avian cells. His work contributed to early ideas regarding the existence of the provirus. Dr. Svoboda provided a stimulating historical perspective on the field during his presentation, and he continued to make insightful comments throughout the meeting. Many attendees commented that meeting Dr. Svoboda was one of the meeting highlights. Dr. Wills provided the perspective of a previous member of the retrovirus field who left to pursue discoveries in the herpesvirus arena. His laboratory was widely regarded as a leading laboratory studying retrovirus assembly. He described the new work in herpes, inspired by lessons learned from retrovirology, about structural transitions that occur in extracellular virions upon heparin sulfate engagement, which prime herpesviruses for cell entry. He also made many salient points regarding the nature of scientific discovery and what contributes to success in the scientific endeavor. Together, the two keynotes added unique perspectives that were appreciated by all and that served to pass on the wisdom of pioneering retrovirologists to new investigators just entering this dynamic field. This conference offers a unique opportunity for interactions of this kind, by highlighting the work of junior investigators in more than 100 short talks while promoting the participation of senior investigators through dynamic question and answer sessions and keynote presentations.

P. Spearman, A. Telesnitsky

The conference featured ten oral sessions, each themed to reflect dynamic developments in retrovirology. Restriction factors, especially TRIM5-α and APOBEC3G/3F, continued their prominence. One prominent finding was the identification by the Welkin Johnson's laboratory of a TRIM-CypA fusion product encoded by pigtailed macaques that is structurally distinct from a similar fusion in owl monkeys. This second incidence of a TRIM-Cyp fusion illustrates the strong selective pressure on host genomes for generating protective alleles against lentiviruses and suggests that TRIM-Cyp fusions provide a particularly good mechanism for protection. The APOBEC session was marked by continued controversy over the role of deamination in restriction versus deaminase-independent restriction. Structural studies of the catalytic domain of APOBEC3G and of the SOCS box region of Vif provided interesting balance.

A session describing late replication events, entitled "Budding and Vpu," highlighted the discovery of tetherin (also known as BST-2 or CD317) as a restriction factor that acts to prevent particle release and is overcome by Vpu. Tetherin's discovery by Stuart Neil in Paul Bieniasz's lab was highlighted by Dr. Neil, followed by John Guatelli describing his independent discovery of this factor and of the down-regulation of cell surface tetherin by Vpu. This discovery

J. Hicks, S. Hughes

will undoubtedly stimulate new investigations into Vpu biology and provide a new area for targeted therapy. Another example of how the host/virus standoff drives genome evolution came from the Accessory Proteins session, which included talks about Vpx suggesting that this previously enigmatic SIV factor also evolved to counter host antiviral responses.

Reflecting an explosion of HTLV biology, this year's meeting featured almost an entire session of groundbreaking talks on HTLV. The HTLV talks covered entry, transcription, new host factor interactions, and animal models of pathogenesis. The Integrase session was one of the most exciting that we have seen at this meeting. New data on LEDGF tethering of preintegration complexes to chromatin and on mapping of functional domains were presented by the Poeschla and Engelman laboratories, as well as others.

Studies of viral assembly maintained a prominent role at this year's meeting. In addition to the budding/Vpu talks, a session on RNA export and assembly highlighted the still evolving story of RNA export roles on the targeting of Gag and viral particle formation. The final session highlighted assembly-related issues and aspects of cell–cell transmission. The transmission of virus from infected macrophages and dendritic cells was featured, as was the mysterious nature of "intracellular" collections of virions in organelles bearing late endosome markers but somehow maintaining continuity with the plasma membrane. The live cell imaging of particle formation was highlighted by Noel Jouvenet from the Bieniasz laboratory, and the final talk featured provocative time-lapse images from the Benjamin Chen lab, suggesting productive transfer of multiple virions en masse from producer to target T cells at junctions called virological synapses. Although slightly underattended, participation in the final session was stronger than in previous years, possibly due to a change in conference dates that resulted in a final Saturday morning session, rather than on Sunday as in previous years.

This was the second year of the annual Andy Kaplan prize that commemorates Dr. Kaplan, a prominent AIDS researcher who died 2 years ago. The prize was won by Dr. Cheoma Okeoma, a junior investigator working in the laboratory of Susan Ross at the University of Pennsylvania. Cheoma presented her work on murine APOBEC3-mediated restriction of MMTV replication in infected animals. Interestingly, the block occurs both from packaged APOBEC3 and from APOBEC3 present within the target cell.

Overall, these oral sessions, paired with the vigorous poster sessions as a forum for extensive discussions, provided the broad exposure to retrovirology that has marked this meeting as the one indispensable yearly meeting for the basic retrovirologist. The meeting continues to provide outstanding science in an intense and critical atmosphere while also providing a great forum for collegial discussions and networking for investigators in the field.

E. Urano, K. Miyauchi

B. Wohrl, J. Tozser, L. Menéndez-Arias, Z. Hanna

PROGRAM

Envelope/Entry/Fusion
Chairpersons: Y. Koyanagi, *Kyoto University, Japan;* M. Johnson, *University of Missouri, Columbia*

Restriction Factors I (TRIM)
Chairpersons: C. Aiken, *Vanderbilt University, Nashville, Tennessee;* T. Hatziioannou, *Aaron Diamond AIDS Research Center, New York*

Pathogenesis/HTLV
Chairpersons: P. Green, *Ohio State University, Columbus;* K. Jones, *SAIC-Frederick, Maryland*

Keynote Address
J. Svoboda, *Academy of Sciences of the Czech Republic*

RNA Export, Assembly
Chairpersons: R. Craven, *Pennsylvania State University College of Medicine, Hershey;* S. Goff, *Columbia University, New York*

Restriction Factors II (APOBEC/Vif)
Chairpersons: S. Sawyer, *University of Texas, Austin;* Y.-H. Zheng, *Michigan State University, East Lansing*

Budding and Vpu
Chairpersons: J. Guatelli, *University of California, San Diego;* S. Neil, *King's College London, United Kingdom*

Transcription, RNA Packaging, RT
Chairpersons: J.-I. Sakuragi, *Osaka University, Japan;* S. Sarafianos, *University of Missouri, Columbia*

Keynote Address
J. Wills, *Pennsylvania State University College of Medicine, Hershey*

Accessory Proteins
Chairpersons: V. Planelles, *University of Utah, Salt Lake City;* C. Transy, *Institut Cochin INSERM U567, Paris, France*

Integration
Chairpersons: D. Grandgenett, *St. Louis University Health Science Center, Missouri;* M. Kvaratskhelia, *Ohio State University, Columbus*

Cell Biology/Cell–Cell Transmission
Chairpersons: A. Ono, *University of Michigan Medical School, Ann Arbor;* L. Wu, *Medical College of Wisconsin, Milwaukee*

K. Hrecka, M. Rits

P. Jolicoeur, G. Towers

Glia in Health and Disease

July 17–21 181 participants

ARRANGED BY **Ben Barres,** Stanford University School of Medicine
Beth Stevens, Children's Hospital/Harvard Medical School

Glial cells constitute 90% of cells in the human nervous system but their roles in the developing adult and diseased brain are still poorly understood. This meeting gathered students and scientists from across the world to discuss their recent progress. In the first part of the meeting, sessions focused on understanding the normal roles of glia, including sessions on glial development, genetic analysis of glial function, myelination, axon–glial interactions, and astrocyte function at synapses and blood vessels. In the last half of the meeting, sessions focused on recent progress on understanding the roles of glia in reactive gliosis, CNS regenerative failure, and disease processes including neurofibromatosis, multiple sclerosis, and encephalitis. Poster sessions were well attended with active discussion. There was again a sense of excitement that our understanding of the function of glia is quickly moving forward, with glia having central roles in normal brain development, function, and disease. This excitement was reflected in the presence of several top journal editors interested in enhancing their coverage of latest advances concerning glia.

B. Stevens, S. John

J. Dugas, B. Barres

T. Sakurai, N. Takada

PROGRAM

Genetic Analysis of Glial Function
Chairpersons: R. Jackson, *Tufts University School of Medicine, Boston, Massachusetts;* K. McCarthy, *University of North Carolina, Chapel Hill*

Gliovascular Interactions
Chairpersons: M. Nedergaard, *University of Rochester, New York;* B. MacVicar, *University of British Columbia, Vancouver*

Myelinating Cells
Chairpersons: M. Simons, *Max-Planck Institute for Experimental Medicine, Göttingen, Germany;* W. Macklin, *Cleveland Clinic, Ohio*

Glial Development
Chairpersons: C. Klambt, *University of Munster, Germany;* M. Wegner, *University of Erlangen-Nürnberg, Germany*

Axon–Glial Interactions
Chairpersons: A. Lloyd, *University College London, United Kingdom;* K. Nave, *Max-Planck Institute of Experimental Medicine, Göttingen, Germany*

Glial Function at Synapses
Chairpersons: S. Duan, *Chinese Academy of Sciences, Shanghai;* C. Eroglu, *Duke University Medical Center, Durham, North Carolina*

Microglia and Gliosis
Chairpersons: M. Sofroniew, *University of California, Los Angeles;* S. Rivest, *Université Laval, Canada*

Glia and Disease
Chairpersons: R. Reynolds, *Imperial College London, United Kingdom;* P. Lowenstein, *Cedars-Sinai Medical Center, Los Angeles, California*

K. Tanaka, C. Eroglu

Caricaturist at work

Mechanisms and Models of Cancer

August 13–17 430 participants

ARRANGED BY **Dafna Bar-Sagi,** New York University Medical Center
Ronald DePinho, Dana-Farber Cancer Institute
Jacqueline Lees, MIT Center for Cancer Research
Charles Sherr, HHMI/St. Jude's Children's Research Hospital

Molecular alterations in tumor-suppressor genes and oncogenes and their associated pathways and networks contribute in a very significant way to the development of human cancers. The last several years have seen an enormous increase in research dealing with cancer genes and their roles in growth control and various stages of tumor development. This second meeting focused on a variety of topics related to cancer genetics, biology, and therapy, reflecting an increased emphasis on integrated approaches to studying signaling in normal and cancer cells, as well as the use of sophisticated in vivo mouse models to study various aspects of cancer biology. The meeting emphasized new discoveries and provided an open forum for the presentation of the latest research and results on different aspects of research on molecular mechanisms and cell and animal models of cancer. The meeting featured two fascinating keynote talks on different aspects of cancer biology by Scott Lowe (HHMI/Cold Spring Harbor Laboratory) and Celeste Simon (HHMI/University of Pennsylvania), two poster sessions, and eight oral sessions. This meeting was enthusiastically supported, and the lectures and poster presentations led to extensive discussions and exchanges of information and ideas.

C. Sherr, D. Bar-Sago, J. Lees, R. DePinho

This conference was funded, in part, by the National Cancer Institute, a branch of the National Institutes of Health. The meeting will be held again in the early fall of 2010.

PROGRAM

Stem Cells and Organismal Development
Chairpersons: T. Jacks, *HHMI/Massachusetts Institute of Technology, Cambridge;* S. Morrison, *University of Michigan, Ann Arbor*

Keynote Address
S. Lowe, *HHMI/Cold Spring Harbor Laboratory*

Mouse Models of Cancer
Chairpersons: G. Lozano, *University of Texas M.D. Anderson Cancer Center, Houston;* T. van Dyke, *National Cancer Institute, Frederick, Maryland*

Signaling Mechanisms
Chairpersons: J. Settleman, *MGH Cancer Center, Harvard Medical School, Boston, Massachusetts;* R. Shaw, *Salk Institute for Biological Studies, La Jolla, California*

Experimental Therapeutics
Chairperson: W. Weiss, *University of California, San Francisco*

Sharing informal notes

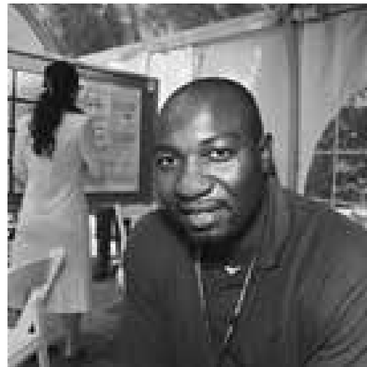
Microenvironment, Inflammation, and Metastasis
Chairpersons: K. Polyak, *Dana-Farber Cancer Institute, Boston, Massachusetts;* Z. Werb, *University of California, San Francisco*

Keynote Address
M.C. Simon, *HHMI/University of Pennsylvania*

Senescence and Apoptosis
Chairpersons: L. Attardi, *Stanford University, California;* M. Oren, *Weizmann Institute of Science, Rehovot, Israel*

Cancer Genetics and Epigenetics
Chairpersons: L. Chin, *Harvard Medical School, Boston, Massachusetts;* D. Felsher, *Stanford University School of Medicine, California*

DNA Damage and Cell Cycle Checkpoints
Chairperson: H. Piwnica-Worms, *HHMI/Washington University, St. Louis, Missouri*



E. Boamah

D. Rebatchouk, L. Beverly

C. Gorrini, M. Wilhelm

Molecular Genetics of Bacteria and Phages

August 20–24 181 participants

ARRANGED BY **Sarah Ades,** Penn State University
Gary Dunny, University of Minnesota Medical School
James Hu, Texas A&M University

This meeting featured 74 oral presentations and 79 poster presentations. Bacteria and phages continue to provide critical insights into diverse areas of biology, ranging from ecology, evolution, and diversity, to gene expression and development, to infectious diseases. The meeting featured exciting presentations covering all of these topics, along with some changes in the organization of th meeting to emphasize different aspects of major biological problems, many of these involving interactions among bacteria, phages, and their ecological niches. A highlight of the meeting was the inclusion of a keynote lecture preceding the banquet by Alan Grossman (Massachusetts Institute of Technology), who provided an enlightening and entertaining presentation that included a wonderful mixture of personal insights and remembrances of his previous Cold Spring Harbor meetings, along with an exciting presentation of his current studies of cell–cell signaling and horizontal gene transfer in *Bacillus subtilis*. The opening session focused on cell biology, division, and development. The first lecture describing regulation of the FtsZ ring assembly and its role in division of *B. subtilis* was presented by Richard Weart (Massachusetts Institute of Technology), who recently completed his Ph.D. at Washington University with Petra Levin and was the 2008 recipient of the Sternberg Thesis Prize for the best Ph.D. thesis in Prokaryotic Molecular Genetics. Other presentation topics in this session included the molecular mechanisms of cell lysis and division, initiation of spore coat assembly, and additional studies of the coordination between chromosome replication and cell division. The ability of microbes to sense and respond to various forms of stress and damage to cellular components is essential to their continued survival, and two oral sessions were devoted to this general topic. The many interesting presentations in this area included a description of the increase in genetic diversity associated with long-term starvation by Steve Finkel (University of Southern California) and the interplay among antibiotic-induced stress, cell–cell signaling, and programmed cell death in *Escherichia coli* by Hanna Engelberg-Kulka (Hebrew University). Stress and DNA damage also can increase mutagenesis, recombination, and lateral gene transfer, as elucidated by the presentations of Susan Rosenberg (Baylor) and John Chen (New York University). Both bacterial pathogenesis and phage biology were included in the host/parasite interaction session, where the presentations ranged from a wonderful “chalk talk” on flagellar assembly by Kelly Hughes (University of Utah) to a description of the mechanisms by which the recently discovered CRISPR sequences protect bacteria from phage infection by Matthijs Jore (Wageningen University). One session of the conference was

A. Grossman, S. Ades

K. Gibbs, H. Engelberg-Kulka

devoted to the mechanisms of environmental sensing and cell–cell signaling, including presentations by Malcolm Winkler (Indiana University) on the essential role of a two-component signal transduction system in pathogenic *Streptococcus pneumoniae* and Karine Gibbs (University of Washington), who discussed the genetic basis for self-recognition in swarming colonies of Proteus. A traditional major focus of the phage meeting has been the microbial RNA world, and this year's conference was no exception, with three sessions devoted to transcription initiation, elongation, termination, and processing, as well as translational control and small RNAs as regulators and effectors of genetic mobility. Highlights of these sessions included presentations on the interplay between Group II intron mobility and global bacterial regulators by Marlene Belfort (Wadsworth Center, New York Department of Health), the role of CRP in regulation of transcription of toxin genes by George Munson, and elucidation of the conformation and dynamics of the RNA polymerase clamp using FRET technology by Richard Ebright (HHMS, Rutgers), which kicked off a fantastic final session.

This meeting was funded, in part, by the National Science Foundation.

W. Szybalski, B. Stillman

PROGRAM

The Nat L. Sternberg Award
Introduction: D. Siegele, *Texas A&M University;* Richard B. Weart, *Massachusetts Institute of Technology*
Regulation of FtsZ Ring Assembly and Cell Division in *B. subtilis*

Cell Biology, Division, and Development
Chairperson: T. Bernhardt, *Harvard Medical School, Boston, Massachusetts*

Environmental Stress, Toxins, and Antitoxins
Chairperson: S. Finkel, *University of Southern California, Los Angeles*

DNA Recombination, Transfer, and Damage Control
Chairperson: J. Peters, Cornell University, Ithaca, New York

Host/Parasite Interactions
Chairperson: A. Salyers, *University of Illinois, Urbana*

RNA Processing and RNA Regulation
Chairperson: M. Belfort, *Wadsworth Center, Albany, New York*

Regulation of Transcription: DNA-binding Proteins
Chairperson: D. Siegele, *Texas A&M University, College Station*

Sensing and Adaptation
Chairperson: M. Winkler, *Indiana University, Bloomington*

Keynote Address
Chairperson: A.D. Grossman, *Massachusetts Institute of Technology*

Transcription from Initiation to Termination, Pausing Along the Way
Chairperson: R. Ebright, *HHMI/Rutgers University, Piscataway*

G. Dunny, B. Buttaro

R. Taylor

K. Hughes

Nuclear Receptors and Disease

August 27–31 160 participants

ARRANGED BY **Ronald Evans,** The Salk Institute
Sohaib Khan, University of Cincinatti
Charles Sawyers, HHMI/Memorial Sloan-Kettering Cancer Center
Keith Yamamoto, University of California, San Francisco

Research on nuclear hormones and nuclear hormone receptors has long proceeded along a broad front. Classical endocrinological studies revealed the first nuclear hormones, their physiological functions, and the clinical sequelae of deficiency and excess, whereas the receptors were first detected via chemical synthesis of a radiolabeled hormone, described in biochemical experiments, genetically manipulated and functionally analyzed using molecular biological procedures and structurally defined by NMR and X-ray crystallography. These studies yielded basic information about eukaryotic transcription, produced the most heavily prescribed therapeutics as well as "reverse endocrinology" approaches to the discovery of ligands, and provided insights into development and disease. This biennial conference brings together students and established investigators—basic and clinical, from academia, pharma, and biotech—to share and integrate ideas, concepts, and approaches across this broad field. This exciting meeting, second in the series, unveiled the structure of a full-length heterodimeric receptor; two novel classes of compounds that may combat cancer; new determinants of selectivity and specificity of receptor action; a pathway for control of stem cell proliferation; networks that link circadian cycling, metabolism, energy use and life span; and new coregulatory factors that either focus or diversify receptor-mediated regulatory mechanisms. The next Cold Spring Harbor Nuclear Receptor meeting is planned for the fall of 2010.

K. Yamamoto

PROGRAM

Keynote Address
K. Yamamoto, *University of California, San Francisco*

Thyroid Hormones and Retinoids: Mechanisms, Physiology, and Pathology
Chairperson: B. Vennström, *Karolinska Institute, Solna, Sweden*

Corticosteroids: Mechanisms, Physiology, and Pathology
Chairperson: J. Funder, *Prince Henry's Institute of Medical Research, Clayton, Australia*

PPARs: Mechanisms, Physiology, and Pathology
Chairperson: D. Kelly, *Burnham Institute for Medical Research, La Jolla, California*

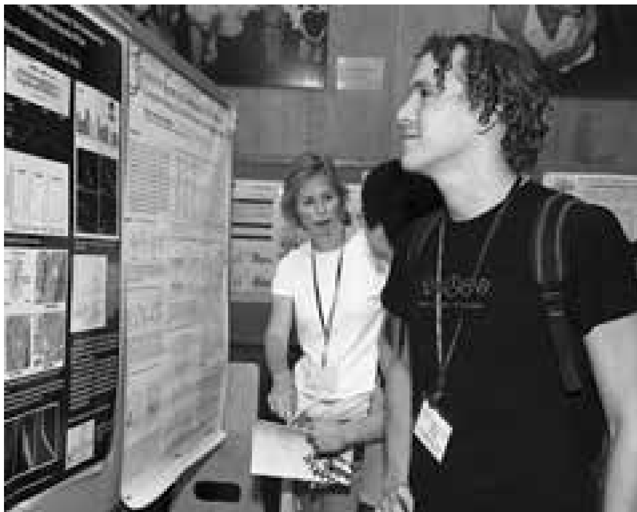
Nuclear Receptor Cofactors: Mechanisms, Physiology, and Pathology
Chairperson: B. O'Malley, *Baylor College of Medicine, Houston, Texas*

F. Claessens, L. Nagy

Estrogens and Progestins: Mechanisms, Physiology, and Pathology
Chairperson: S. Fuqua, *Baylor College of Medicine, Houston, Texas*

Androgens: Mechanisms, Physiology, and Pathology
Chairperson: M. Brown, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Nuclear Receptors in Invertebrates: Development, Metabolism, and Life Span
Chairperson: D. Mangelsdorf, *University of Texas Southwestern Medical Center, Dallas*



W. Zwart

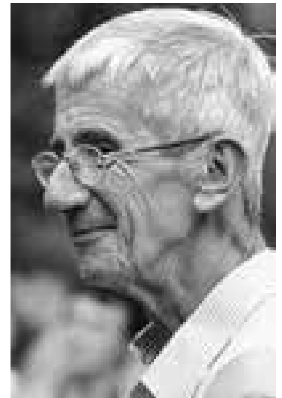
Translational Control

September 3–7 443 participants

ARRANGED BY **Alan Hinnebusch,** National Institute of Child Health & Human Development/NIH
Terri Kinzy, UMDNJ R.W. Johnson Medical School
Peter Sarnow, Stanford University

This meeting attracted more than 440 participants from around the world and included Joan Steitz as an invited speaker, eight platform sessions, and three poster sessions that covered 360 abstracts. Novel findings on the mechanism of translation included high-resolution structures of ribosomal complexes at different stages of translation. Cryo-EM analysis of a bacterial preinitiation complex showed fMet-tRNA and initiation factors IF1 and IF2 bound to the 30S subunit. Cryo-EM structures of pre-translocation 70S complexes were presented that support the hybrid states model, with tRNAs in hybrid positions between the A and P sites and P and E sites before translocation. A cryo-EM structure of *Escherichia coli* ribosomes in a complex with EF-Tu and aminoacyl-tRNA revealed how GTP hydrolysis is induced by correct codon reading. Ribosomal structures were presented showing the nascent peptide in the exit tunnel of a translating ribosome.

There were several breakthroughs regarding soluble translation factors. In mammalian mitochondria, the separate functions of EF-G in elongation and ribosome recycling were assigned to separate, albeit homologous, proteins: HMEFG1 catalyzes tRNA movement but is inactive in ribosome recycling, whereas HMEFG2 promotes ribosome recycling but is inactive in elongation. Mitochondrial IF2 was shown to contain an additional insert enabling it to replace both IF1 and IF2 in *E. coli* cells. NMR studies led to a model of the eukaryotic scanning complex in which eIF4G positions the helicase eIF4A at the mRNA entry channel to unwind secondary structure in advance of the ribosome. The RNA-binding protein YB-1 was shown to impose a requirement for PABP in forming the activated eIF4F-mRNP complex. Yeast eIF3 subunits a, b, and j were implicated in scanning and AUG recognition, and distinct elements in eIF1A were identified with opposing effects on scanning. A new RNA helicase DHX29 was shown to be required for scanning on structured 5′ leaders, and it was reported that eIF5 acts as a GDP dissociation inhibitor (GDI) beyond its GTPase-activating function for eIF2. Mouse eIF6 was shown to stimulate translation in vivo, and the enigmatic factor eIF5A was found to enhance elongation in yeast cells.


R. Jackson

Important findings on translational control elements in mRNA included a crystal structure of the cricket paralysis virus IRES mimicking tRNA in a P/E hybrid state, explaining how it bypasses all initiation factors. The eIF4G homolog DAP5 was shown to bind and stimulate cellular IRES elements in

O. Fayet, M. Altmann, M. Hentze

CDK1 and Bcl-2 mRNAs. The 3′ UTR of VEGF-A mRNA was found to contain a GAIT element whose repressive function in interferon-treated cells is overridden in hypoxia by binding of hnRNP L. Identification of new mRNA targets of ribosomal protein L13a has expanded GAIT-controlled mRNAs into a posttranscriptional operon. Regarding microRNAs, snoRNAs were found to be novel sources of microRNAs in humans and protozoans, and it was shown that the miR-34 family is induced by p53/DNA damage and targets c-*myc* mRNA. It was shown that deadenylation contributes to translational repression by microRNAs in *Drosophila* cell-free extracts and in mammalian cells. microRNAs were observed to activate translation in quiescent serum-starved cells and *Xenopus* oocytes.

In the arena of translational control in disease, there is now strong evidence for virus-driven evolution of the antiviral eIF2α kinase PKR. It appears that eIF4GI overexpression promotes inflammatory breast cancer by enhancing IRESs in mRNAs that promote tumor cell survival, angiogenesis, and metastasis. It was reported that human cytomegalovirus (HCMV) induces PABP translation, and ICP27 of herpes simplex virus-1 likely recruits PABP to stimulate viral mRNA translation. Novel eIF2α kinases were identified in the parasite *Toxoplasma gondii* that regulate developmental stages and cellular stress responses.

New insights into connections between translation and mRNA turnover or localization were also reported. There is evidence that mRNA decay takes place in yeast while the mRNA is still engaged in translation. Among proteins that associate with the stress granule (SG) factor G3BP, p63 was found in association with initiation factors, and the ubiquitin-specific protease USP10 was shown to be required for SG but not P-body formation. SG components TIA-1 and TIAR were implicated in repressing translation of 5′TOP mRNAs during amino acid starvation. There is evidence that sequential phosphorylation and dephosphorylation of zipcode-binding protein (ZBP) represses actin mRNA translation in neurons until it is transported to distal neurites and growth cones.

This meeting was funded, in part, by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health.

PROGRAM

Decay and Subcellular Structures
Chairperson: J. Lykke-Andersen, *University of Colorado, Boulder*

Factors and Mechanisms
Chairperson: G. Pavitt, *University of Manchester, United Kingdom*

Elongation and Termination
Chairperson: M. Rodnina, *University of Witten/Herdecke, Germany*

Development, CNS, and Signaling
Chairperson: C. Bagni, *Catholic University of Leuven, Belgium*

miRNAs and Regulation of Factors
Chairperson: T. Preiss, *Victor Chang Cardiac Research Institute, Sydney, Australia*

Ribosome Structure and Function
Chairperson: M. Yusupov, *IGBMC, Illkirch, France*

Viruses and Disease
Chairperson: E. Harris, *University of California, Berkeley*

mRNA Regulatory Elements
Chairperson: M. Holcik, *Children's Hospital of Eastern Ontario, Ottawa, Canada*

T. Pe'ery, N. Sonenberg

M. Gupta, K. Kleene

Axon Guidance, Synaptogenesis, and Neural Plasticity

September 10–14 415 participants

ARRANGED BY **Anirvan Ghosh,** University of California, San Diego
Christine Holt, University of Cambridge
Alex Kolodkin, Johns Hopkins University

C. Holt, A. Kolodkin, A. Ghosh

The human brain has billions of nerve cells (neurons) and each neuron is typically connected to hundreds of other neurons via synapses in a highly precise fashion. This complex neural wiring underlies the ability of humans, and other animals, to interact with the outside world, to learn and to perform complex behaviors. The complexity of the brain, in fact, surpasses that of a computer, and one of the major challenges for the field of neuroscience is to understand how nerve connections are made accurately and reliably. Connections between different regions of the brain are first established during embryogenesis and result in the formation of a stereotyped pattern of axon pathways or fiber tracts. Projection neurons are often a long distance away from their synaptic targets, and the first step in establishing connectivity is to extend an axon tipped with a growth cone. The growth cone is the sensory/motile apparatus responsible for steering an accurate course through the early neuroepithelium of the embryonic brain. What directs the navigation of growth cones along the correct pathway? Once the growth cone reaches its target area, it must then find the correct region to terminate and begin to make synapses. How do axons select the correct cells with which to make synapses? Finally, after synapses are initially made, they can be modified by activity and experience, leading to "plastic" changes that are important in key processes such as learning and memory. What determines whether a synapse is maintained or lost? How are these plastic changes controlled? This meeting focused on these key issues in axon guidance, synaptogenesis, and plasticity.

L. Luo, N. Gray

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 and genetic approaches in vertebrates and in invertebrates have led to mutually reinforcing discoveries that have helped to 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 sixth of these meetings involved sessions devoted to particular problems in the assembly of the nervous system, with speakers chosen from among those submitting abstracts by Session chairs who are leaders in the field. Other abstracts were presented as posters.

Like the previous five meetings, the response of the field to the 2008 conference was one of overwhelming enthusiasm: There were about 415 participants, 285 of whom submitted abstracts; 60 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, molecular, dynamic imaging, biochemical, and genetic). In addition, there were two keynote addresses: Professor Carla Shatz (Stanford) reviewed the history of the field of the development of retinogeniculate and geniculocortical connections and included some of the seminal contributions made by her own group, and Professor Paul Forscher (Yale University) focused on the cytoskeletal dynamics in the growth cone and showed spectacular time-lapse movies of actin and microtubules illuminating the mechanics of growth. The meeting provided an important forum for ideas and approaches and helped scientists in the field obtain the most up-to-date information, enabling them to meet, network, and establish collaborations. At the previous meeting, a need was identified for an additional poster session because the number and quality of abstracts submitted for posters were so high. Therefore, this year a third poster session was introduced that was extremely well attended, and this session went some way toward alleviating problems with overcrowding. Based on the enthusiastic comments of the participants, the intensity of the oral and poster sessions, and the large crowds that stayed 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 for Neurological Diseases and Stoke, a branch of the National Institutes of Health, and Merck Research Laboratories.

PROGRAM

Axon Guidance I: Guidance Molecules
Chairpersons: A. Chedotal, *CNRS, Université Paris 6, France;* D. Van Vactor, *Harvard Medical School, Boston, Massachusetts*

Synapse Formation I
Chairpersons: Y. Jin, *University of California, San Diego;* G. Davis, *University of California, San Francisco*

Special Lecture: Tuning Up Circuits: Brain Waves, Immune Genes, and Synapse Plasticity
C. Shatz, *Stanford University, California*

Neural Circuits and Plasticity
Chairpersons: B. Dickson, *Institute of Molecular Pathology, Vienna, Austria;* M. Crair, *Yale University, New Haven, Connecticut*

Axon Guidance II: Dynamics and Targeting
Chairpersons: L. Luo, *Stanford University, California;* M. Tessier-Lavigne, *Genentech Inc., South San Francisco, California*

Neuronal Polarity and Migration
Chairpersons: E. Stoeckli, *University of Zurich, Switzerland;* F. Polleux, *University of North Carolina, Chapel Hill*

Axon Guidance III: Transcriptional, Translational Control/Regeneration
Chairpersons: C. Mason, *Columbia University, New York;* Y. Zou, *University of California, San Diego*

Synapse Formation and Dendritic Patterning
Chairpersons: K. McAllister, *University of California, Davis;* P. Salinas, *Imperial College London, United Kingdom*

Special Lecture
P. Forscher, *Yale University, New Haven, Connecticut*

Axon Guidance IV: Signaling and Guidance
Chairpersons: L. Richards, *University of Queensland, Australia;* S. Pfaff, *The Salk Institute, La Jolla, California*

Wine and cheese

Dynamic Organization of Nuclear Function

September 17–21 294 participants

ARRANGED BY **Genevieve Almouzni,** Institut Curie, France
David Spector, Cold Spring Harbor Laboratory
Susan Wente, Vanderbilt University Medical Center, Tennessee

This meeting focused on the relationships between nuclear structure and function. The opening session highlighted current studies on gene expression and genome function, with the session chair (Andy Belmont) giving a beautiful introduction to the session highlighting the topic areas to be covered and critical questions relating to models of chromosome structure. He transitioned to describe his own studies using cell lines and mouse ES cells expressing tagged BAC transgenes to microscopically monitor chromatin structure during differentiation. Of great interest and debate were talks focused on new methods to pinpoint the molecular mechanisms and intranuclear organization underlying transcriptionally active versus inactive chromatin. The following Thursday morning's session, chaired by John York, was a new addition to the program and focused on nuclear signaling. With a novel chalk-talk approach, York introduced the audience to the field of soluble inositol signaling and its impact on nuclear physiology. This session highlighted the roles for signaling in regulating transcription, mRNA export, and chromatin structure. Signaling from or at the nuclear envelope also revealed novel mechanisms for influencing nuclear envelope morphology, breakdown, and cell differentiation. After a lively poster session and wine and cheese party, Tom Misteli chaired the dynamics of chromatin, DNA replication, and repair session.

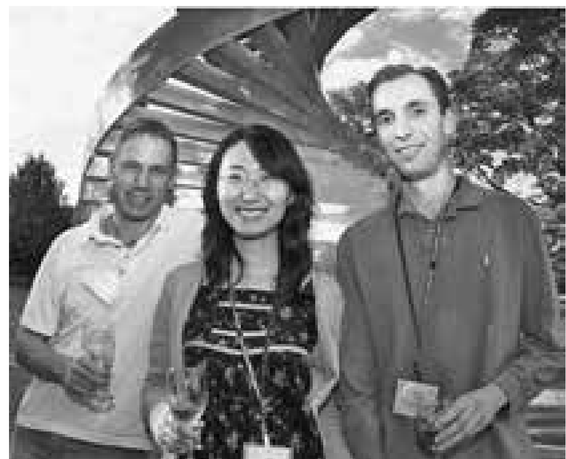
S. Wente, D. Spector, G. Almouzni

The work presented illustrated the two faces of chromatin dynamics, that of the chromatin fiber and of chromatin-binding proteins involved in replication and repair. A lively discussion ensued concerning the programming of DNA replication origins and their specificity. Friday morning started with the nuclear domains and bodies session chaired by Sui Huang. This has been a long-standing topic at the meeting; however, this year, the talks illustrated the tremendous progress that is being made in analyzing the

Through the looking glass: Poster session in Bush Auditorium

mechanisms of assembly for nuclear bodies and their functional roles. Lunch was followed by an afternoon session on epigenetics and nuclear organization chaired by Wendy Bickmore. This session revisited in more depth topics of genome control and renewed the ongoing and lively debate about repressive versus activating chromatin environments within the nucleus. An evening poster session ended the day, with discussions at the posters continuing for many more hours than anticipated. The nucleocytoplamic transport session started Saturday morning and was chaired by Ueli Aebi who gave a broad introduction to the nuclear pore complex and transport mechanisms. The session included vigorous discussions of the competing models for facilitated transport, as well as reports of new factors required for mRNA export, tRNA shuttling, and pore complex assembly. New evidence was also presented for changes in pore permeability and nuclear leakiness with cell differentiation and age. The afternoon session on chromosomes and the cell cycle was ably chaired by Rebecca Heald and described the regulation of mitotic chromosome dynamics as well as the roles for nuclear transport factors in cell cycle progression. The final Sunday morning session on nuclear structure and disease was chaired by Wim Vermeulen and gave multiple direct examples of how the machinery involved in nuclear organization and function is linked to the underpinnings of several pathophysiologic states. This included a subgroup of talks on the newest insights into altered A-type lamin function in the premature aging disease Progeria, as well as talks probing the molecular determinants of cancer-prone symptoms of xeroderma pigmentosum and Cockayne syndrome, of the fatal bone marrow failure syndrome dyskeratosis congentia, and of the Roberts and Cornelia de Lange developmental syndromes.

The enthusiasm of the meeting participants was overwhelming, and it is remarkable how with each successive meeting, the interest has grown and strengthened. It is anticipated that the CSHL 2010 Symposium will focus on "Functional Organization of the Cell Nucleus." On the basis of success of the past meetings, the major insights in the field, and its expansion, one can anticipate that the Symposium will be a great success!



M. Huebner, R. Zhao, J. Wilusz

O. Cohen-Fix

N. Watkins

PROGRAM

Gene Expression and Genome Function
Chairperson: A. Belmont, *University of Illinois, Urbana, Champaign*

Nuclear Signaling
Chairperson: J. York, *HHMI/Duke University Medical Center, Durham, North Carolina*

Dynamics of Chromatin, DNA Replication, and Repair
Chairperson: T. Misteli, *NCI/National Institutes of Health, Bethesda, Maryland*

Nuclear Domains and Bodies
Chairperson: S. Huang, *Northwestern University School of Medicine, Chicago, Illinois*

Epigenetics and Nuclear Organization
Chairperson: W. Bickmore, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

Nucleocytoplasmic Transport
Chairperson: U. Aebi, *University of Basel, Switzerland*

Chromosomes and the Cell Cycle
Chairperson: R. Heald, *University of California, Berkeley*

Nuclear Structure and Disease
Chairperson: W. Vermeulen, *Erasmus University, Rotterdam, The Netherlands*

Biotechnology: Past, Present, and Future

September 21–23 91 participants

ARRANGED BY **Mila Pollock** and **Jan Witkowski,** Cold Spring Harbor Laboratory

ADVISORS **Sydney Brenner**
Peter Feinstein
Lee Hood
Tom Maniatis
Richard Roberts

Biotechnology is a science that dates back to the fermentation and breeding practices of the ancient Egyptians. The discoveries of Pasteur and Mendel in the 19th century furthered this scientific field. Today we recognize the developments of the latter part of the 20th century as the origins of the modern biotechnology era. The application of recombinant DNA technologies sparked the "Biotech Revolution" and ushered in the rapid growth of a new industry. This special conference was supported and arranged by the Laboratory's Genentech Center for the History of Molecular Biology and Biotechnology. The conference explored the growth of a new industry from its creation to the extraordinary impact it has had on our quality of life, and it attracted prominent industry participants to examine how the scientific and business worlds came together and how they will continue to impact the industry's future. These scientists, entrepreneurs, and venture capitalists—all of whom were essential to the industry's growth—were joined by the sociologists, economists, and historians who have analyzed the extraordinary impact the products of the biotech industry have had on our lives. Sessions at the meeting included talks and panel discussions addressing a variety of themes.

K. Murray, C. Weissmann

M. Van Montagu

PROGRAM

Origins of Biotechnology
Keynote Addresses
R. Roberts, **Biotechnology: Past, present, and future**
R. Bud, **Prehistory: From ancient times to 1970**
Stelios Papdopoulos, **Business and modern biotech: 1970–1980**

Early Days in Biotechnology
C. Weissmann, *Biogen*
H. Heyneker, *Genentech*

Enabling Technology
D. Comb, *Role of support companies*
M. Feldman, *Commercializing Cohen-Boyer: The origins of university biotechnology transfer*
P. Feinstein, *Venture investing in human therapeutics*

Interactions of Biotechnology and Society
R. Steinbrook, *Influence of government policies*
A. McHughen, *Influence of public: Genetically modified organisms*
J. Leamon, *DNA sequencing and personal genomes*

The Raw Data: Biotechnology Documentation
K. Thibodeau, *The survival and exploitation of scientific data in cyberspace*

Panel Discussion: Preserving and Accessing the History of Biotechnology
Chairpersons: R. Bud, S. Grefshein, and M. Pollock

Lessons for the Future
G. Bohlin, *Starting a biotech company in the 21st century*
Y.-L. Lo, *Emerging markets: Perspectives from China presented by Julie Xing*
M. Van Montagu, *Biotechnology: Economics and the developing world*

Panel Discussion: Promoting the History of Biotechnology
Chairpersons: K. Murray, R. Olby, R. Roberts, D. Tobell, and J. Witkowski

N. and K. Murray

R. Roberts, K. Murray

Molecular Genetics of Aging

September 24–28 279 participants

ARRANGED BY **Judith Campisi,** Lawrence Berkeley National Laboratory
David Sinclair, Harvard University
Steven Austad, University of Texas Health Science Center

Aging is controlled by genetics and the environment and leads to biological changes that put organisms at increasing risk for a panoply of diseases and eventual death. Remarkable progress has been made in recent years in establishing a molecular foundation for understanding how genes control and influence aging and how genetic and environmental manipulations can alter its course. This conference provided an in-depth forum for new findings and concepts in current aging research and stimulated lively discussions on how genetic pathways interact with each other and the environment to influence aging in diverse organsims. Two sessions focused specifically on genetics. One highlighted the growing list of genetic pathways that influence life span in simple model organisms such as nematodes and fruit flies, and the other focused on conserved and nonconserved pathways that modulate aging and life span in mammals. Two related session focused on how genes that promote somatic genome stability postpone aging and age-related diseases, and how cellular responses to genotoxic and other forms of stress drive aging phenotypes. A session was devoted to understanding how mitochondrial function and nutrient sensing and metabolism influence aging and the relationship between these processes and longevity. Two sessions focused on cell proliferation. One highlighted processes that maintain proliferative homeostasis in mitotic and postmitotic tissues, and another explored the role of stem cells in the aging of specific tissues. Finally, one session focused on how the environment interacts with genes to influence aging, and on recent advances in develop-

S. Austad, J. Campisi, D. Sinclair

R. Taylor, H. Aguilaniu, N. Erjavec

J. Witkowski, E. Blackburn

ing environmental interventions in the aging process. The conference captured the excitement surrounding the rapid progress that is being made in understanding the molecular bases for aging, and highlighted the many important questions that have yet to be answered in this growing field.

This meeting was funded, in part, by the National Institute on Aging, a branch of the National Institutes of Health.

PROGRAM

Genetics (Simple Organisms)
Chairpersons: H. Tissenbaum, *University of Massachusetts Medical School, Worcester;* S. Pletcher, *Baylor College of Medicine, Houston, Texas*

Genomic Stability/Damage/Repair/Telomeres
Chairpersons: J. Vijg, *Albert Einstein College of Medicine, Bronx, New York;* E. Blackburn, *University of California, San Francisco*

Oral Presentations from Abstracts
S. Austad, *University of Texas Health Science Center, San Antonio;* J. Campisi, *Lawrence Berkeley National Laboratory, Buck Institute for Age Research, California;* D. Sinclair, *Harvard Medical School, Boston, Massachusetts*

Mitochondria/Metabolism
Chairpersons: P. Rabinovitch, *University of Washington, Seattle;* L. Guarente, *Massachusetts Institute of Technology, Boston*

Cellular Responses: Senescence/Apoptosis/Stress
Chairpersons: J. Sedivy, *Brown University, Providence, Rhode Island;* N. Sharpless, *University of North Carolina, Chapel Hill*

Stem Cells
Chairpersons: I. Conboy, *University of Berkeley, California;* K.L. Rudolph, *University of Ulm, Germany*

Proliferative Homeostasis
Chairpersons: P. Hasty, *University of Texas Health Science Center, San Antonio;* R. Bodmer, *Burnham Institute for Medical Research, La Jolla, California*

Environment/Interventions
Chairpersons: R. Miller, *University of Michigan, Ann Arbor;* S. Spindler, *University of California, Riverside*

Genetics (Complex Organisms)
Chairpersons: A. Brunet, *Stanford University, California;* J. Hoeijmakers, *Erasmus University, Rotterdam, The Netherlands*

L. Guarente, S. Michan

B. Stillman, E. Blackburn

N. Sharpless, S. Primmer

Germ Cells

October 1–5 218 participants

ARRANGED BY **Anne Ephrussi,** EMBL, Germany
Renee Reijo Pera, Stanford University School of Medicine
David Zarkower, University of Minnesota

This meeting began with a perspective on the female germ line and reprogramming by Dr. J. Gurdon, with acknowledgement of the significant role that Dr. Ann McLaren has had over many years in unraveling the mysteries of the germ cell. Notably, Dr. Gurdon compared the reprogramming strategies and efficiencies from different organisms. Although commonly thought to be inefficient, somatic cell nuclear transfer takes advantage of the oocyte and has a remarkable efficiency that can reach up to 30%–50% in some organisms. The relationship between reprogramming by the oocyte and the production of induced pluripotent stem cells remains elusive.

The emphasis on reprogramming continued with a discussion of novel aspects of the oocyte-to-embryo transition. In particular, Dr. H. Ueno demonstrated his system to track the birth of germ cells and the size of the founding population. Dr. M. Yao presented data demonstrating that the mammalian *Oct4* gene is required in the oocyte-to-embryo transition, and Drs. Chen and Seydoux discussed the regulatory activities of CDK-1 in activating MBK-2 during the oocyte-to-embryo transition in *Caenorhabditis elegans*.

There has been remarkable progress since the last Germ Cells meeting (in 2006) in the area of germ cell specification in diverse organisms from planeria to zebra fish, mice, and humans. Indeed, the use of embryonic stem cells (both mouse and human) to differentiate germ cells for genetic analysis was the key focus in a number of talks and poster presentations. Issues that have slowed progress were addressed including markers for isolation of germ cells and

E. Huebner, C. Extavour

M. McGovern, F. Marlow

P. Western, J. Gurdon

strategies to provide the niche. Numerous genes, in vivo, that are required for germ cell specification have been identified and are well characterized including *Bucky ball* and *Nanos2* (and homologs).

Posttranslational mechanisms of regulation and development were featured as knowledge in this area grows and the importance of posttranslational mechanisms becomes abundantly more clear. Indeed, from specification to proliferation and maintenance to differentiation, posttranslational mechanisms have important roles in regulating transcript abundance, availability, and protein modifications.

The next Germ Cells meeting will be held in the fall of 2010 and will be organized by M. Fuller, J. Eppig, and P. Newmark.

The 2008 meeting was funded, in part, by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health and Labor Foundation, and the March of Dimes.

PROGRAM

Themes in Germ Cell Biology
Chairperson: S. Strome, *University of California, Santa Cruz*

Reprogramming and the Oocyte-to-embryo Transition
Chairperson: S. DiNardo, *University of Pennsylvania School of Medicine, Philadelphia*

Germ Cell Specification and Patterning
Chairperson: J. Kimble, *HHMI/University of Wisconsin, Madison*

Germ Cell Formation In Vivo and In Vitro
Chairperson: R.S. Hawley, *Stowers Institute for Biomedical Research, Kansas City, Missouri*

Germ Line Stem Cells
Chairperson: R. Lehmann, *HHMI/New York University Medical Center, New York*

Epigenetic Regulation
Chairperson: A. Surani, *University of Cambridge, United Kingdom*

Posttranscriptional Regulation of Germ Cell Development
Chairperson: Y. Saga, *National Institute of Genetics, Mishima, Japan*

Germ-line Programs
Chairperson: A. Spradling, *HHMI/Carnegie Institute, Baltimore, Maryland*

Caricaturist

D. Page, J. Mueller

Personal Genomes

October 9–12 168 participants

ARRANGED **Richard Gibbs,** Baylor College of Medicine
Mary-Claire King, University of Washington
Maynard Olson, University of Washington
Lincoln Stein, Cold Spring Harbor Laboratory
Jan Witkowski, Banbury Center, Cold Spring Harbor Laboratory

This special meeting was arranged both to celebrate and to critically examine a significant milestone in human genetics—the first "personal genomes." Ultra-high-throughput sequencing strategies are used in a very limited number of laboratories and few scientists, and even fewer clinical geneticists, are familiar with the implications of the "$1000" genome. This meeting provided an opportunity to explore a number of important themes and a panel discussion on the ethical, legal, and social implications (ELSI) of these rapidly changing technologies. The critical success and timeliness of the meeting can be judged from the measurable enthusiasm of the audience and the appearance of two major editorials citing the conference in considerable depth in subsequent issues of *Nature* magazine. The collective decision was taken to hold the meeting for a second time at Cold Spring Harbor in the fall of 2009.

L. Stein, G. Weinstock

F. Collins, H. Sussman

R. Myers, E. Mardis

PROGRAM

Introduction: Setting the Tone
J.D. Watson, *Cold Spring Harbor Laboratory*

Single Genome Sequences

Making Sense of the Content of Whole Genomes

Panel Discussion: Ethics
Chairperson: F. Collins, *National Human Genome Research Institute, Bethesda, Maryland*

Applications Of Whole-genome Studies

Whole-genome Genetics

New Developments

W.R. McCombie, J. Watson

C. Bustamente, O. Bahcall

M. Banck, A. Butler

Mouse Genetics and Genomics: Development and Disease

October 29–November 2 186 participants

ARRANGED BY

Kathryn Anderson, Memorial Sloan-Kettering Cancer Center
Hiroyuki Sasaki, National Institute of Genetics
William Skarnes, Wellcome Trust Sanger Institute
Anthony Wynshaw-Boris, University of California School of Medicine, San Francisco

This meeting is held every other year at Cold Spring Harbor Laboratory. Researchers from across the globe from very diverse areas of biology 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.

A. Wynshaw-Boris, K. Anderson

The meeting was organized into eleven sessions and included the Annual Rosa Beddington Lecture given by Janet Rossant. Each session was arranged around two to three talks of general interest, given by both senior and 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 meeting also included the presentation of nearly 100 posters in two afternoon sessions. It ended with a farewell to Hiro Sasaki and Tony Wynshaw-Boris, who stepped down after 4 years as organizers of the meeting. They will be replaced by Michael Shen and Haruhiko Koseki.

H. Sasaki

Some of the sessions have shifted in their emphasis during the past 2 years. The growing emphasis on genetics/genomics and on human disease models, already noted in previous years, has also continued, leading to the change in title this year. 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 year, neurogenetic diseases were a focal point of the session featuring mouse models of human diseases. Due to the rapid development of mouse genetic resources, there was also a mouse ES cell workshop headed by Bill Skarnes.

This meeting was funded, in part, by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health.

PROGRAM

Germ Cells and Stem Cells
Chairperson: H. Schoeler, *Max-Planck Institute for Molecular Biology, Münster, Germany*

Organogenesis
Chairperson: F. Costantini, *Columbia University College of Physicians & Surgeons, New York*

Neurogenetics
Chairperson: S. McConnell, *Stanford University, California*

Epigenetics
Chairperson: H. Koseki, *RIKEN Research Center for Allergy and Immunology, Yokohama, Japan*

Patterning
Chairperson: D. Kingsley, *Stanford University, California*

Genetics and Genomics
Chairperson: S. Orkin, *HHMI/Children's Hospital, Boston, Massachusetts*

Mouse ES Workshop
Chairperson: W. Skarnes, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Rosa Beddington Lecture: Cell Lineage and Cell Fate in the Early Embryo
J. Rossant, *The Hospital for Sick Children, Toronto*

Human Disease Models
Chairperson: J. Crawley, *NIMH/National Institutes of Health, Bethesda, Maryland*

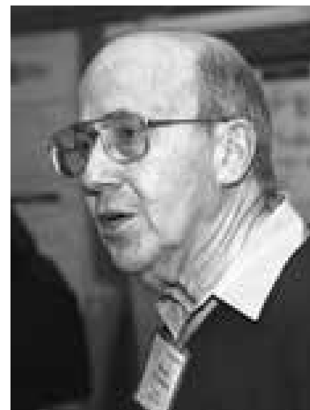
S. McConnell, J. Partanea

W. Skarnes

Pharmacogenomics

November 19–22 147 participants

ARRANGED BY **Michel Eichelbaum,** Institute of Clinical Pharmacology, Germany
Steve Leeder, Children's Mercy Hospital, Kansas City
Munir Pirmohamed, University of Liverpool
Dick Weinshilboum, Mayo Medical School, Minnesota
Scott Weiss, Brigham and Women's Hospital
Roland Wolf, University of Dundee



R. Weinshilboum

This sixth meeting, a joint project of CSHL and the Wellcome Trust, was funded, in part, by the National Institute of General Medical Sciences (NIGMS), one of the NIH institutes. This series of meetings, held in alternating years on the CSHL and Sanger Centre campuses, is designed to bring together scientists from disciplines that range from basic genomics to clinical medicine who are studying the role of inheritance in variation in drug response phenotypes—phenotypes that can range from life-threatening adverse drug reactions to lack of the desired therapeutic drug effect. As a result, pharmacogenomics represents a major component of the movement toward "personalized" or "individualized" medicine. The opening session began with a scientific presentation by Jeremy Berg, Director of NIGMS, followed by a presentation from Scott Weiss, Chair of the NIH-sponsored, multi-institutional Pharmacogenetics Research Network (PGRN), and a presentation highlighting the scientific accomplishments of the Foundation NIH (FNIH) by Dr. Stephen Spielberg, a member of the FNIH Board. A major theme running through the meeting was the explosive growth of the application to pharmacogenomics of genome-wide techniques such as genome-wide association studies (GWAS) and "next-generation" DNA sequencing. Sessions devoted to cardiovascular pharmacogenomics and adverse drug reactions were complemented by sessions that focused on pharmacogenomic GWAS, bioinformatics, and developments in DNA sequencing, with a presentation on next-generation sequencing by Rick Wilson, Director of the Washington University Genome Center. The PGRN sponsored a session on pharmacogenomic mechanisms that included presentations on genome-wide studies of enhancer elements by Eddie Rubin and the pharmacogenomics of drug therapy of congestive heart failure by

J. Ramatoulie, M. Pirmohamed

S. Leeder, K. Aitcheson

Stephen Liggett. The major theme that emerged from the meeting was the convergence of genomic and pharmacogenomic science as we move toward a more highly individualized use of drugs in the treatment and/or prevention of disease.

PROGRAM

Opening Session
Chairpersons: R.M. Weinshilboum, *Mayo Clinic, Rochester, Minnesota;* J.S. Leeder, *Children's Mercy Hospital, Kansas City, Missouri*

Whole-genome Associations and Translational Bioinformatics
Chairpersons: M. Eichelbaum, *Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany;* C.R. Wolf, *University of Dundee, United Kingdom*

Basic Genomics: DNA Sequencing and CNV
Chairpersons: M. Pirmohamed, *University of Liverpool, United Kingdom;* M. Eichelbaum, *Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany*

Cardiovascular Pharmacogenomics
Chairpersons: D. Roden, *Vanderbilt University, Nashville, Tennessee;* A. Shuldiner, *University of Maryland School of Medicine, Baltimore*

Advances in Pharmacogenomics of Adverse Drug Reactions
Chairpersons: M. Pirmohamed, *University of Liverpool, United Kingdom;* J.S. Leeder, *Children's Mercy Hospital, Kansas City, Missouri*

Mechanisms in Pharmacogenomics
Chairpersons: K. Giacomini, *University of California, San Francisco;* S. Liggett, *University of Maryland, Baltimore*

Clinical Application of Pharmacogenomics and Public-private Partnerships
Chairpersons: R. Valdes, *University of Louisville, Kentucky;* T. Moyer, *Mayo Clinic, Rochester, Minnesota*

K. Skoglund, A. Snapir

Y. Garten

R. LaFond, E. Woodahl

Blood Brain Barrier Physiology

December 1–4 67 participants

ARRANGED BY **Roland J. Bainton,** University of California, San Francisco
Ben Barres, Stanford University

The aim of this new Cold Spring Harbor Winter Biotechnology Conference was to encourage new conceptual approaches and novel methods to our understanding of the neuroprotective physiologies of blood brain barrier (BBB) structures, pairing genetics of model organisms and associated biological methods with recent insights into vertebrate BBB physiology and development. The conference fostered cross-disciplinary exchange of ideas and expertise among developmental and evolutionary biologists, vascular and BBB physiologists, and disease-oriented industry scientists interested in modifying or circumventing specific pathways of neuroprotection. Keynote addresses on different aspects of BBB physiology were given by Tom Reese and Rich Daneman.

As is traditional at Cold Spring Harbor meetings, selection of material for oral and poster presentation was made by the organizers and individual session chairs on the basis of scientific merit.

R. Bainton, R. Daneman

B. Barres on closed circuit television

N. Saunders

PROGRAM

Imaging and Modeling Blood Brain Barrier during Health and Disease
Chairpersons: M. Nedergaard, *University of Rochester, New York;* A. Prat, *University of Montreal, Canada*

Molecular Constituents of Blood Brain Barrier
Chairpersons: G. Beitel, *Northwestern University, Evanston, Illinois;* M. Furuse, *Kobe University, Japan*

Keynote Address
R. Daneman, *Stanford University, California*

Cellular Physiology of Blood Brain Barrier
Chairpersons: A. Armulik, *Karolinska Institute, Stockholm, Sweden;* C. Klambt, *University of Münster, Germany*

VI Screening
Chairpersons: I.E. Blasig, *Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany;* J. Dow, *University of Glasgow, United Kingdom*

Transport and Drug Delivery
Chairpersons: B. Sarkadi, *Hungarian Institute of Science, Budapest;* R. Watts, *Genentech Inc., South San Francisco, California*

Keynote Address
T. Reese, *NINDS/National Institutes of Health*

Molecular Regulation of Blood Brain Barrier
Chairpersons: U. Gaul, *The Rockefeller University, New York;* C.J. Kuo, *Stanford University, California*

G. Galvis, A. Armulik

G. Murray, S. Cohen, H. Stolp, P. Johannson

D. Miller reviewing posters

Neurodegenerative Diseases: Biology and Therapeutics

December 4–7 123 participants

ARRANGED BY **Sam Gandy,** Mount Sinai School of Medicine
Virginia Lee, University of Pennsylvania School of Medicine
Marcy MacDonald, Massachusetts General Hospital

As many as one-half of those aged 85 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 human diseases as well as some of the hallmark molecular and morphological pathology of the conditions.

V. Lee, D. Stewart

Rational biochemical and cell-based screens have generated lead compounds that show promise in living animal models. Most importantly, the animal models have enabled discovery of entirely unanticipated therapeutic strategies (such as amyloid β immunotherapy). Some of these rationally discovered compounds and unexpected immunotherapies are now in phase II and III 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 this meeting, eight 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 evening meals.

Topics in the 2008 meeting included federal and foundation perspectives on neurodegenerative drug discovery, presymptomatic evaluation, imaging methodology development, amyloid imaging in humans and mouse models, synthesizing protein evidence from imaging, cell biology, and genetics in order to approach sporadic neurodegenerative diseases, genetic, biochemical, and physiological markers; clinical trials; innovative trial design; modulation of disease by aging, metabolic, excitotoxic and extracellular matrix factors; protein conformational considerations (oligomerization); and the frontotemporal dementia–motor neuron disease–parkinsonism spectrum of disease. Diseases considered at this meeting included Alzheimer’s, Huntington’s, ALS, prion diseases, Parkinson’s, tauopathies, and synu-

M. MacDonald

cleinopathies, as well as the newly described TDP-43-related diseases. A session was also devoted to an update on the Alzheimer's disease neuroimaging initiative, and another session was devoted to genome-wide association studies of several major neurodegenerative diseases.

This meeting was funded, in part, by Merck Research Laboratories.

T. Klaber, P. Jaeger

PROGRAM

NIA Alzheimer's Disease Neuroimaging Initiative (ADNI) Update

Biomarkers in Alzheimer's Disease

Genetic Approach to Target Discovery (GWAS)

Talks from Submitted Abstracts

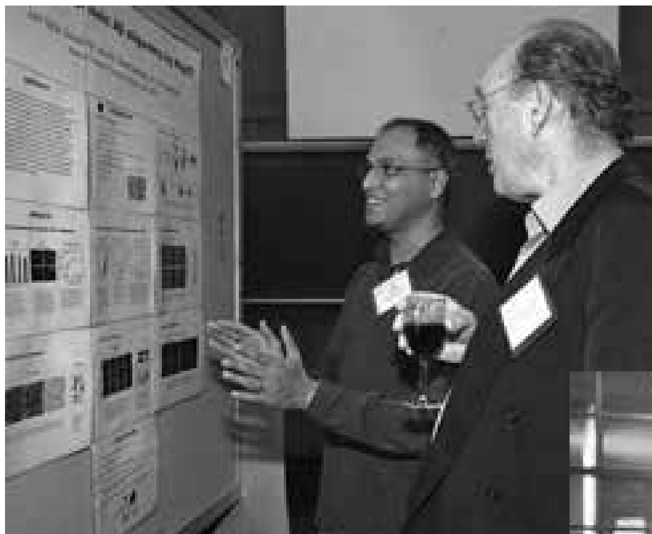
Amyotrophic Lateral Sclerosis

Huntington's Disease and Dyt1 Dystonia

Update on Targets and Drug Discovery in Alzheimer's I

Update on Targets and Drug Discovery in Alzheimer's II

J. Witkowski, J. Trojanowski



A. Cachikar, C. Masters

M. Fahnestock, S. Gandy

Engineering Principles in Biological Systems

December 10–13 73 participants

ARRANGED BY

Hana El-Samad, University of California, San Francisco
Partha Mitra, Cold Spring Harbor Laboratory
Richard Murray, California Institute of Technology
Christina Smolke, California Institute of Technology

The first meeting on Engineering Principles in Biological Systems was held in December 2006, following an earlier planning workshop at the Banbury Conference Center in May of the same year. At that meeting, attendees expressed appreciation of the unique and broad scope of the meeting, with the aim to repeat the meeting in December 2008. The two key ideas behind this meeting were that theoretical principles which have been developed in the context of human engineered systems are useful in understanding biological function and that these principles apply across scales, from the cellular to the organismal level. In keeping with these ideas, the meeting attracted researchers in a broad range of fields, ranging from bacterial systems biology to neural systems, with shared interest in engineering principles. Sessions were broken up according to broad areas of engineering, including a general introduction to the field, and individual sessions on multicellular communities, evolution, intracellular networks, and synthetic biology/theory. There was considerable enthusiasm among the group for continuing this unique meeting series, and a third meeting will be held in Cambridge, United Kingdom in fall 2009, as part of the CSHL/Wellcome Trust joint conference program.

P. Mitra, R. Rabadan

This meeting was funded, in part, by the Center for Biological Circuit Design (CBCD) at the California Institute of Technology.

Group photo

PROGRAM

Introduction
P. Mitra, *Cold Spring Harbor Laboratory*

Multicellular Communities
Chairperson: H. El-Samad, *University of California, San Francisco*

Neurobiology
Chairperson: S. Schiff, *Pennsylvania State University, University Park*

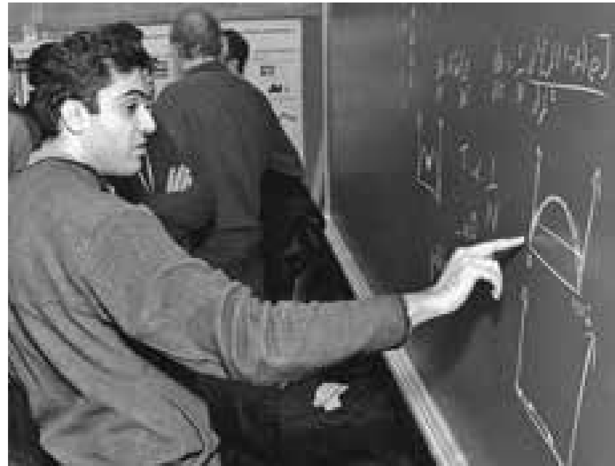
Evolution
Chairperson: L. Caporale, *New York, New York*

Intracellular Networks I
Chairperson: R. Murray, *California Institute of Technology, Pasadena*

Intracellular Networks II
Chairperson: R. Murray, *California Institute of Technology, Pasadena*

Synthetic Biology and Theory
Chairperson: C. Smolke, *California Institute of Technology, Pasadena*

R. Greenspan



M. Atwal

B. Mishra, R. Rabadan

M. Donahue, K. Galloway

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 teach 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 2–15

INSTRUCTORS
K. Adelman, National Institutes of Health/NIEHS, Research Triangle Park, North Carolina
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
M. Marr, Brandeis University, Waltham, Massachusetts

ASSISTANTS
S. Duellman, University of Wisconsin, Madison
D. Gilchrist, NIEHS, Research Triangle Park, North Carolina
B. Glaser, University of Wisconsin, Madison
Y.-C. Lee, M.D. Anderson Cancer Center/University of Texas, Houston
S. Nechaev, NIEHS, Research Triangle Park, North Carolina
L. Nutt, Duke University Medical Center, Durham, North Carolina
N. Thompson, University of Wisconsin, Madison
W. Turki-Judeh, University of California, Los Angeles
K. Wright, University of California, Berkeley
J. Zamudio, University of California, Los Angeles

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 discussion session every evening. Students 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 *Escherichia coli*, and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques included precipitation by salts, pH, and ionic polymers; ion exchange, gel filtration, hydrophobic interaction, and reverse phase chromatography; lectin affinity, ligand affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis, and electroblotting; and high-performance liquid chromatography. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were used 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. In addition to the non-CSHL instructors, the course also included CSHL investigator Leemor Joshua-Tor.

This course was supported with funds provided by the National Cancer Institute (http://www.cancer.gov/).

PARTICIPANTS

Block, G., M.S., Tulane University Health Sciences Center, New Orleans, Louisiana
Chen, H., Ph.D., Washington University, St. Louis, Missouri
Folch, E., Ph.D., IIBB-CSIC, Barcelona, Spain
Foronjy, R., M.D., Columbia University, New York
Jalili, R., M.S., Stanford Genome Technology Center, Palo Alto, California
Jendresen, C., M.S., Technical University of Denmark, Lyngby
Kimura, M., Ph.D., Gifu University School of Medicine, Gifu, Japan
Kraft, E., Ph.D., Monsanto, Davis, California
Lindemos, S., M.S., University of Copenhagen, Denmark
Lu, J., M.Sc., The University of Warwick, Coventry, United Kingdom
McLain, T., B.S., Colorado State University, Fort Collins
Santiago, R.-G., Ph.D., University of British Columbia, Vancouver, Canada
Smith, J., B.A., University of Utah, Salt Lake City
Spivak, A., B.S., Washington University in St. Louis School of Medicine, Missouri
Wang, M., B.S., University of Texas Southwestern Medical Center, Dallas
Zanic, M., Ph.D., Max-Planck Institute, Dresden, Germany

SEMINARS

Adelman, K., NIH/NIEHS, Research Triangle Park, North Carolina: RNA polymerase is poised for development across the genome.
Burgess, R., University of Wisconsin, Madison: Introduction to protein purification, immunoaffinity chromatography.
Burgess, R. and Glaser, B., University of Wisconsin, Madison: RNA polymerase-sigma factor interactions and use of LRET-based assays for drug discovery and biochemistry.
Courey, A., University of California, Los Angeles: Multiple roles for SUMO in cell biology and development.
Joshua-Tor, L., Cold Spring Harbor Laboratory: DNA translocation: One step at a time.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Adhesion molecules and osteogenesis in prostate cancer bone metastasis.
Marr, M., Brandeis University, Waltham, Massachusetts: Controlling gene expression in response to extracellular signals.

Cell and Developmental Biology of *Xenopus*

April 5–15

INSTRUCTORS	**R. Keller,** University of Virginia, Charlottesville **K. Kroll,** Washington University School of Medicine, St. Louis, Missouri
ASSISTANTS	**T. Nakayama,** University of Virginia, Charlottesville **D. Shook,** University of Virginia, Charlottesville

Xenopus is the leading vertebrate model for the study of gene function in development. The combination of lineage analysis, gene-knockout strategies, experimental manipulation of the embryo, and genomic/bioinformatic techniques made it ideal for studies on the molecular control of embryo patterning, morphogenesis, and organogenesis. The course combined intensive laboratory training with daily lectures from recognized experts in the field. Students learned both emerging technologies and classical techniques to study gene function in *opus* development. An important element was the informal interaction between students and course faculty. Technologies covered included oocyte and embryo culture, lineage analysis, and experimental manipulation of embryos; time-lapse imaging of morphogenesis; gain- and loss-of-function analyses using mRNAs and antisense oligos; whole-mount in situ hybridization, immunocytochemistry, RT-PCR, and genomic/bioinformatic techniques; preparation of transgenic embryos; and use of *Xenopus tropicalis* for genetic analyses. This course was designed for those new to the *Xenopus* field, as well as those wanting a refresher course in the emerging technologies. The course was open to investigators from all countries.

Lecturers and assistants for the 2008 course included Ken Cho (University of California, Irvine), Jan Christian (Oregon Health and Sciences University), Rob Grainger (University of Virginia), Richard Harland (University of California, Berkeley), Janet Heasman (Cincinnati Children's Hospital Research

Foundation), Mustafa Khokha (Yale University School of Medicine), Paul Krieg (University of Arizona College of Medicine), Takuya Nakayama (University of Virginia), and Christopher Wylie (Cincinnati Children's Hospital Research Foundation).

This course was supported with funds provided by the National Institute of Child Health and Human Development (http://www.nichd.nih.gov/), the National Science Foundation (http://www.nsf.gov/), and the Howard Hughes Medical Institute (http://www.hhmi.org/).

PARTICIPANTS

Battu, G., Ph.D., National Cancer Institute at Frederick, Frederick, Maryland
Beyer, T., Dipl., University of Hohenheim, Stuttgart, Germany
Corsing, A., M.S., Martin-Luther University Halle/Saale, Germany
Harding, J., M.B., Cancer Research, London, United Kingdom
Hindley, C., M.B., University of Cambridge, Cambridge, United Kingdom
Janssens, S., M.S., VIB-Ghent University, Zwijnaarde, Belgium
Lutz, I., Ph.D., Leibniz-Institute of Freshwater Ecology and Inland, Berlin, Germany
Mantecca, P., Ph.D., University of Milano Bicocca, Milano, Italy
Miller, R., Ph.D., University of Texas/M.D. Anderson Cancer Center, Houston
Powell, W., Ph.D., Kenyon College, Gambier, Ohio
Richardson, W., B.Sc., Medical Research Council, Edinburgh, United Kingdom
Schmucker, D., Ph.D., Harvard Medical School and Dana-Farber Cancer Institute, Boston, Massachusetts
Wang, F., M.Sc., Cancer Research UK, Cambridge, United Kingdom
Weber, T., Dipl., University of Hohenheim, Stuttgart, Germany
Widder, P., B.S., Virginia Polytechnic Institute and State University, Blacksburg
Williams, R., B.Sc., University of East Anglia, Norwich, United Kingdom

SEMINARS

Cho, K., University of California, Irvine: Cell adhesion and tissue assembly during *Xenopus* morphogenesis.
Christian, J., Oregon Health and Science University, Portland: TGF-β signaling during early embryogenesis.
Grainger, R., University of Virginia, Charlottesville: Transcriptional analysis of lens induction.
Harland, R., University of California, Berkeley: Pattern by the organizer.
Heasman, J., Children's Hospital Medical Center, Cincinnati, Ohio: Maternal control of *Xenopus* development.
Keller, R., University of Virginia, Charlottesville: Cell behavior during early morphogenesis.
Khokha, M., Yale University, New Haven, Connecticut: *X. tropicalis* and genetic analysis of development.
Krieg, P., University of Arizona College of Medicine, Tucson: Postgastrula organogenesis.
Kroll, K., Washington University School of Medicine, St. Louis, Missouri: Using transgenic *Xenopus* to study transcriptional regulation.
Wylie, C., Children's Hospital Research Foundation, Cincinnati, Ohio: *Xenopus* as a model system.

Workshop on Schizophrenia and Related Disorders

June 4–13

INSTRUCTORS **A. Malhotra,** Zucker Hillside Hospital, Glen Oaks, New York
D. Porteous, University of Edinburgh, United Kingdom

ASSISTANTS **J. Barnett,** Massachussets General Hospital, Boston
J. Hall, University of Edinburgh, Edinburgh, United Kingdom

This workshop provided students with the most current understanding of the molecular, cellular, and neural systems underlying disturbances in brain function in these devastating illnesses. During the 10-day workshop, students learned about the clinical aspects of schizophrenia, schizoaffective disorder, and bipolar disorder and explored in detail the genetic and neurobiological underpinnings of these complex psychiatric disorders. The Workshop was divided into four main sessions: Clinical overview, Genetics and genomics, Developmental neurobiology and neural circuits, and Cognitive systems. In addition to hearing about the most recent research in these areas, controversial topics and challenges to basic assumptions in the field were explored and discussed. A diverse faculty brought the most up-to-date results and theories to the students, making this workshop a valuable resource for young researchers starting out in this fast-moving and expansive field. Not only did it help them to build the foundation for their future research, it also introduced them to many potential collaborators working to understand schizophrenia and similar disorders from different perspectives.

This workshop was supported with funds provided by the Oliver Grace Fund.

PARTICIPANTS

Adams, A., B.S., University of Prince Edward Island, Charlottetown, Canada
Austin, B., B.S., University of Georgia, Athens
Baker, B., B.S., University of Maryland, College Park
Bolstad, I., B.S., University of Oslo, Oslo, Norway
Carlyle, B., B.S., Edinburgh University, Edinburgh, United Kingdom
Charych, E., Ph.D., Wyeth Research, Princeton, New Jersey
DeRosse, P., B.S., Zucker Hillside Hospital, Glen Oaks, New York
Di Forti, M., Ph.D., Institute of Psychiatry, London, United Kingdom
Eliasen, G., B.S., University of Aarhus, Aarhus, Denmark
Ellman, L., Ph.D., Columbia University, New York
Foster, K., Ph.D., Merck & Co, Inc., West Point, Pennsylvania
Gamo, N., B.S., Yale University, New Haven, Connecticut
Hamilton, J., B.S., Howard University, Washington, D.C.
Karlsgodt, K., Ph.D., University of California, Los Angeles
Kirtley, A., B.S., Cardiff University, United Kingdom
Labrie, V., B.S., University of Toronto, Canada
Linderholm, K., B.S., Karolinska Institute, Solna, Sweden
Oh, S.W., Ph.D., Allen Institute for Brain Science, Seattle, Washington
Stuetz, A., Ph.D., Johns Hopkins University, Baltimore, Maryland
Tillmann, C., B.S., Max-Planck Institute for Brain Research, Frankfurt, Germany
Trotman, H., B.S., Emory University, Atlanta, Georgia
Zilles, D., Ph.D., Georg-August University Goettingen, Germany

SEMINARS

Abi-Dargham, A., Columbia University, New York: Dopamine system.
Austin, C., National Institutes of Health, Bethesda, Maryland: High-throughput screening and chemical genomics: Toward new drug development for schizophrenia.
Barch, D., Washington University, St. Louis, Missouri: The cognitive neuroscience of schizophrenia.
Barnett, J., Massachusetts General Hospital, Boston: Introduction; genetics primer.
Benes, F., Harvard University, Cambridge, Massachusetts: Deconstructing the trisynaptic pathway as a strategy for defining cellular endophenotypes of schizophrenia and bipolar disorder.
Cornblatt, B., The Feinstein Institute for Medical Research, North Shore Long Island Jewish Health System, Manhasset, New York: The schizophrenia prodrome.
Cross, A., AstraZeneca: Translational approaches, part II.
Deisseroth, K., Stanford University, California: Probing circuit function.
Egan, M., Merck & Co, Inc., North Wales, Pennsylvania: Translational approaches, part 1.
Grace, A., University of Pittsburgh, Pennsylvania: Summary of neurobiology; neuroanatomical and neurophysiological underpinnings of schizophrenia.
Hall, J., University of Edinburgh, United Kingdom: Genetic imaging in schizophrenia; introduction; genetics primer.
Killcross, S., Cardiff University, United Kingdom: Cognitive models of schizophrenia.
Krystal, J., Yale University, New Haven, Connecticut: Glutamate system/GABA.
Law, A.J., University of Oxford, United Kingdom: The neuregulin story.
Lehner, T., National Institutes of Mental Health, Bethesda, Maryland; Heimer, H., Schizophrenia Forum, Providence, Rhode Island; Brose, K., Neuron, Cambridge, Massachusetts; Kramer, G., NARSAD, Great Neck, New York: Publishing, grants, resources for schizophrenia research.
Lewis, D., University of Pittsburgh, Pennsylvania: Cortical GABA systems in schizophrenia.
Li, B., Cold Spring Harbor Laboratory: Glutamatergic synapse and its receptors: From cognitive function to psychiatric disorder.
Malholtra, A., Zucker Hillside Hospital, Glen Oaks, New York: Pharmacogenetics; innovating target testing through industry-academic partnerships; overview of psychiatric genetics.
McCombie, D., Cold Spring Harbor Laboratory: Next-generation sequencing.
McIntosh, A., University of Edinburgh, United Kingdom: Neuroimaging in psychosis.
Murray, R., Institute of Psychiatry, London, United Kingdom: History and clinical description; neurodevelopment and schizophrenia.
Owens, D., University of Edinburgh, United Kingdom: Clinical phenomenology/neuropharmacology.
Petryshen, T., Massachusetts General Hospital and the Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge: Mouse models of schizophrenia.
Porteous, D., University of Edinburgh, United Kingdom: DISC1 genetics and biology.
Ross, C., Johns Hopkins University, Baltimore, Maryland: Integrating neurodegenerative diseases with schizophrenia.
Sebat, J., Cold Spring Harbor Laboratory: Structural variation.
Sklar, P., Stanley Center, Massachusetts Institute of Technology, Cambridge: Genomic approaches.
Susser, E., Columbia University, New York: Epidemiology of schizophrenia: New directions.
Vawter, M., University of California, Irvine: Gene expression In schizophrenia.
Weinberger, D., National Institutes of Mental Health, Rockville Pike, Maryland: Intermediate phenotypes and schizophrenia-associated genes.

Advanced Bacterial Genetics

June 4–24

INSTRUCTORS **J. Kirby,** University of Iowa, Iowa City
S. Lovett, Brandeis University, Waltham, Massachusetts
A. Segall, San Diego State University, California

ASSISTANTS **J. Guilinger,** Brandeis University, Waltham, Massachusetts
J. Klebensberger, University of New South Wales, Sydney, Australia
G. Perez, San Diego State University, California

This course presented logic and methods used in the genetic dissection of complex biological processes in diverse bacteria. Laboratory methods included classical mutagenesis using transposons, mutator strains, and chemical and physical mutagens; detection and quantitation of gene expression changes using various reporter genes and real-time PCR; the mapping of mutations using genetic and physical techniques; modern approaches to the generation and analysis of targeted gene disruptions and fusions using PCR genetic recombination and cloning methods; epitope insertion mutagenesis; site-directed mutagenesis; and fluorescence microscopy. Key components of the course were the use of sophisticated genetic methods in the analysis of model bacteria (including *Escherichia coli, Salmonella, Bacillus subtilis,* and *Myxococcus xanthus*) and the use of the wealth of new genomic sequence information to motivate these methods.

Invited lecturers presented various genetic approaches to study bacterial mechanisms of metabolism, development, and pathogenesis. Speakers in the 2008 course included Sarah Ades (Penn State University), Lionello Bossi (Centre National Recherche Scientifique), Valerie De Crecy-Lagan (University of Florida), Robert Edwards (San Diego State University), Thierry Emonet (Yale University), Don Ennis

(University of Louisana), Kelly Hughes (University of Utah), Beth Lazazzera (University of California, Los Angeles), Stanley Maloy (San Diego State University), Dianne Newman (Massachusetts Institute of Technology), and Molly Schmid (Keck Graduate Institute). The course admits 16 students, both foreign and U.S. residents, from diverse backgrounds and career levels for intensive (but fun) instruction in microbial genetics. For further academic information about the course, feel free to contact the instructors via their home pages.

This course is supported with funds provided by the National Science Foundation (http://www.nsf.gov/).

PARTICIPANTS

Bednarz, M., M.S., University of Illinois Urbana Champaign, Urbana
Brigati, J., Ph.D., Maryville, College, Tennessee
Buceta, J., Ph.D., Barcelona Science Park, Spain
Coleman, M., B.A., Massachusetts Institute of Technology, Cambridge
De Vooght, L., M.S., Institute of Tropical Medicine, Antwerp, Belgium
Gaines, J., Ph.D., East Carolina University Brody School of Medicine, Greenville, North Carolina
Ivancic Bace, I., Ph.D., Faculty of Science of Molecular Biology, Zagreb, Croatia
Jonas, K., M.S., Karolinska Institutet, Stockholm, Sweden
Konings, A., B.Sc., University of Otago, Dunedin, New Zealand
Lun, D., Ph.D., Broad Institute of MIT and Harvard, Cambridge, Massachusetts
Meyer, A., Ph.D., Massachusetts Institute of Technology, Cambridge
Nadell, C., B.A., Princeton University, New Jersey
Qimron, U., Ph.D., Harvard Medical School, Boston, Massachusetts
Sathyamoorthy, V., Ph.D., Food & Drug Administration, Laurel, Maryland
Shopsin, B., Ph.D., New York University School of Medicine, Skirball Institute, New York
Singh, B., M.Sc., Uppsala University, Sweden

SEMINARS

Ades, S., Penn State University, University Park, Pennsylvania: Border security: Cell envelope stress responses in gram-negative bacteria.
Bossi, L., Centre de Genetique Moleculaire, CNRS, Gif-sur-Yvette, France: A tale of two prophages.
DeCrecy-Lagard, V., University of Florida, Gainesville: Linking gene and function by comparative genomics: Making sense of the unknowns.
Edwards, R., University of Pennsylvania, Philadelphia: Upscaling from genetics to genomics to metagenomics.
Emonet, T., Yale University, New Haven, Connecticut: Digital and experimental investigation of a model sensory system.
Ennis, D., University of Louisanna, Lafayette: DNA repair as an host-pathogen interaction: Chronic mycobacterial infections increases cancer risk; Identification of a new bacterial pathogen from post-Katrina sediments of New Orleans.
Hughes, K., University of Utah, Salt Lake City: Coupling flagellar gene regulation to assembly.
Lazazzera, B., University of Los Angeles, California: Genetic dissection of biofilm formation by *Bacillus subtilis*.
Maloy, S., San Diego State University, California: Rearrangements of the bacterial chromosome.
Newman, D., Princeton University, New Jersey: From iron oxides to infections: Roles for redox active antibiotics in microbial survival and development.
Schmid, M., Microcide Pharmaceuticals, Inc., Mountain View, California: Crossing genetic studies with pharmaceutical discoveries.

Ion Channel Physiology

June 4–24

INSTRUCTORS **B. Clark,** WIBR, University College London, United Kingdom
J. Diamond, National Institutes of Health, Bethesda, Maryland
M. Farrant, University College London, United Kingdom

CO-INSTRUCTOR **M. Holmgren,** National Institutes of Health/NINDS, Bethesda, Maryland

ASSISTANTS **J. Gruendemann,** University College London, United Kingdom
J. Hardie, Northwestern University
M. Jenkins, Emory University, Atlanta, Georgia
R. Kanichay, University College London, United Kingdom
A. Scimemi, National Institutes of Health/NINDS, Bethesda, Maryland
D. Srikumar, NMU/NINDS/NIH, Bethesda, Maryland
B. Stell, Universite Paris 5, France
K. Vervaeke, University College London, United Kingdom

The primary goal of this course was to investigate, through lectures and laboratory work, the properties of ion channels that allowed neurons to perform their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitters 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. The advantages and disadvantages of each

method, preparation, and recording technique were considered with respect to specific scientific questions. Admissions priority was given to students and postdocs with a demonstrated interest, specific plans, and a supportive environment in which to apply these techniques to a defined problem.

Guest speakers in 2008 included Francisco Bezanilla (The University of Chicago), Joshua Dudman (Janelia Farm Research Campus), Nace Golding (The University of Texas at Austin), Dax Hoffman (NIH/NICHD/LCSN), Jeffry Isaacson (University of California, San Diego), Amy Lee (Emory University School of Medicine), David Linden (Johns Hopkins University School of Medicine), Eve Marder (Brandeis University), Andreas Schaefer (Max-Planck Institut fur Medizinische Forschung), Jesper Sjostrom (University College London), Ivan Soltesz (University of California, Irvine), and Nelson Spruston (Northwestern University).

This course is supported by the Howard Hughes Medical Institute (http://www.hhmi.org/). Scientists from developing countries accepted into this course may be eligible for scholarships provided by the International Brain Research Organization (for details, go to http://www.iac-usnc.org/ fellowships.html).

PARTICIPANTS

Baumgart, J., B.A., University of Virginia, Charlottesville
Beane, W., Ph.D., Forsyth Institute, Durham, North Carolina
Fink, A., M.A., Columbia University, New York
Gleichman, A., B.A., University of Pennsylvania, Philadelphia
Gray, E., B.A., University of California, Los Angeles
MacAskill, A., B.A., University College London, United Kingdom
Mingote, S., Ph.D., Columbia University, New York
Nguyen, T., Ph.D., University of Pittsburgh, Pennsylvania
Schneider, E., Ph.D., Princeton University, New Jersey
Siemens, J., B.S., University of California, San Francisco
Wilbrecht, L., Ph.D., University of California, San Francisco
Woods, G., B.S., University of California, Davis

SEMINARS

Bezanilla, P., The University of Chicago, Illinois: Voltage sensors in Na^+ and K^+ channels.
Clark, B., University College London, United Kingdom: Synaptic integration.
Diamond, J., National Institutes of Health/NINDS, Bethesda, Maryland: Synaptic transmission.
Dudman, J., Janelia Farm Research Campus, Ashburn, Virginia: Hyperpolarization-activated current (I_h).
Farrant, M., University College London, United Kingdom: GABA receptors and inhibition.
Golding, N., The University of Texas, Austin: Intrinsic electrical properties and action potential propagation.
Hoffman, D., National Institutes of Health/NICHD, Bethesda, Maryland: Regulation of dendritic excitability by the activity-dependent trafficking of voltage-gated K^+ channels.
Holmgren, M., National Institutes of Health/NINDS, Bethesda, Maryland: Gating of Na^+ and K^+ channels; Regulation of K^+ channel inactivation.
Isaacson, J., University of California, San Diego: Olfactory microcircuits.
Lee, A., Emory University School of Medicine, Atlanta, Georgia: Deconstructing Ca^{2+} channel signaling complexes.
Linden, D., Johns Hopkins University, Baltimore, Maryland: Glutamate receptors.
Marder, E., Brandeis University, Waltham, Massachusetts: Variability, compensation, homeostasis, and neuromodulation in neuronal networks.
Schaefer, A., MPI fur Medizinische Forschung, Heidelburg, Germany: Inhibition, glutamate receptors, and order discrimination in mice.
Sjöström, J., University College London, United Kingdom: Plasticity and LTP.
Soltesz, I., University of California, Irvine: Activity-dependent plasticity of endocannabinoid signaling.

Molecular Embryology of the Mouse

June 4–24

INSTRUCTORS	**D. Threadgill,** University of North Carolina, Chapel Hill **P. Trainor,** Stowers Institute for Medical Research, Kansas City, Missouri
CO-INSTRUCTOR	**L. Pevny,** University of North Carolina, Chapel Hill
ASSISTANTS	**C. Futtner,** University of North Carolina, Chapel Hill **S. Hutton,** University of North Carolina, Chapel Hill **A. Iulianella,** Stowers Institute for Medical Research, Kansas City, Missouri **J. Rivera-Perez,** University of Massachusetts Medical School, Worcester **L. Sandell,** Stowers Institute for Medical Research, Kansas City, Missouri

This intensive laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse development. Lectures provided the conceptual basis for contemporary research in mouse embryogenesis and organogenesis, whereas laboratory practicals provided an extensive hands-on introduction to mouse embryo analysis. Experimental techniques covered included in vitro culture and manipulation of preimplantation and postimplantation embryos; embryo transfer; culture and genetic manipulation of embryonic stem cells; production of chimeras by embryo aggregation and by ES cell injection; and transgenesis by pronuclear microinjection. In addition, this year's practicals featured increased emphasis on phenotypic analysis of mutants, including techniques of histology, in situ hybridization, immunohistochemistry, skeletal preparation, organ culture, and tissue recombination.

Seminar speakers this year included Alexi Aravin (Cold Spring Harbor Laboratory), Richard Behringer (University of Texas M.D. Anderson Cancer Center), Ali Brivanlou (The Rockefeller University), Blanche Capel (Duke University Medical Center), Frank Costantini (Columbia University, College of Physicians & Surgeons), Oliver Guillermo (St. Jude Children's Research Hospital), Kat Hadjantonakis (Memorial Sloan-Kettering Cancer Center), Gregory Hannon (Cold Spring Harbor Laboratory), Konrad Hochedlinger (Massachusetts General Hospital), Monica Justice (Baylor College of Medicine), Robin Lovell-Badge (MRC NIMR, United Kingom), Terry Magnuson (University of North Carolina, Chapel Hill),

Andras Nagy (Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Canada), David Ornitz (Washington University), Luis Parada (University of Texas Southwestern Medical Center), Janet Rossant (The Hospital for Sick Children), Michael Shen (Columbia University Medical Center), Charles Sherr (HHMI/St. Jude Children's Research Hospital), Philippe Soriano (Fred Hutchinson Cancer Research Center), Michelle Southard-Smith (Vanderbilt University Medical Center), Colin Stewart (National Cancer Institute), Lori Sussel (Columbia University), Shahragim Tajbakhsh (Pasteur Institute, France), Joseph Takahashi (HHMI/Northwestern University), Patrick Tam (Children's Medical Research Institute, Australia), and Richard Woychik (The Jackson Laboratory).

This course is supported with funds provided by the National Cancer Institute (http://www.cancer.gov/).

PARTICIPANTS

Alonso, E., Ph.D. National Centre of Cardiovascular Research, Madrid, Spain
Barili, V., M.S., San Raffaele Scientific Institute Developmental Neurogenetics, Milan, Italy
Chandrasekhar, A., Ph.D., University of Missouri, Columbia
Ferrer Vaquer, A., M.S., University of Freiburg, Germany
Gogilotti, R., B.S., Northwestern University, Chicago, Illinois
Mastracci, T., Ph.D., Columbia University, New York
Prajapati, R., Ph.D., London Research Institute, United Kingdom
Rutschow, D., Dipl., University of Dundee, United Kingdom
Starmer, J., Ph.D., University of North Carolina, Chapel Hill
Tachibana-Konwalski, K., Ph.D., University of Oxford, United Kingdom
Teixeira, C., Ph.D., New York University College of Dentistry, New York
Tondeleir, D., M.S., University of Gent, Belgium
Turunen, H., B.S., University of Turku, Finland
Vestin, A., M.Sc., Stony Brook University, New York

SEMINARS

Aravin, A., Cold Spring Harbor Laboratory: RNAi and mammalian growth control.
Behringer, R., The University of Texas M.D. Anderson Cancer Center, Houston: Gene targeting.
Brivanlou, A., The Rockefeller University, New York: ES cell differentiation/pluripotency.
Capel, B., Duke University Medical Center, Durham, North Carolina: Germ cells, genital ridges, and sex determination.
Costantini, F., Columbia University, New York: Kidney organogenesis.
Guillermo, O., St. Jude Children's Research Hospital, Memphis, Tennessee: Forebrain induction/vasculogenesis/lymphangiogenesis.
Hadjantonakis, K., Memorial Sloan-Kettering Cancer Center, New York: Mouse imaging.
Hannon, G., Cold Spring Harbor Laboratory: RNAi and mammalian growth control.
Hochedlinger, K., Massachusetts General Hospital, Boston: Nuclear reprogramming.
Justice, M., Baylor College of Medicine, Houston, Texas: ENU mutagenesis screens and chromosome engineering.
Lovell-Badge, R., MRC National Institute for Medical Research: Sry, sex determination, and Sox genes.
Magnuson, T., University of North Carolina, Chapel Hill: Epigenetics.
Nagy, A., Samuel Lunenfeld Research Institute, Toronto, Canada: Transgenics and knockouts.
Orntiz, D., Washington University, St. Louis, Missouri: Limb and skeletal biology.
Parada, L., University of Texas Southwestern Medical Center, Dallas: Neuroblastoma cancer tumor.
Pevny, L., University of North Carolina, Chapel Hill: Neural stem cells neurogenesis.
Rivera-Perez, J., University of Massachusetts Medical School, Worcester: Early postimplantation.
Rossant, J., The Hospital for Sick Children, Toronto, Canada: Chimeras, ES cell lineages, vasculogenesis.
Shen, M., Columbia University Medical Center, New York: RNAi prostate stem cells.
Sherr, C., HHMI/St. Jude Children's Research Hospital, Memphis, Tennessee: Tumorigenesis, p53, Arf.
Soriano, P., Fred Hutchinson Cancer Research Center, Seattle, Washington: Gene traps and PDGF/Eph/Ephrin signaling.
Southard-Smith, M., Vanderbilt University, Nashville, Tennessee: Enteric nervous system, neural crest, and gut development.
Stewart, C., National Cancer Institute, Frederick, Maryland: ES cells.
Sussell, L., Columbia University, New York: Pancreas organogenesis.
Tajbakhsh, S., Pasteur Institute, Paris, France: Myogenesis and muscle satellite stem cells.
Takahashi, J., HHMI/Northwestern University, Evanston, Illinois: Behavioral genetic screens and development.
Tam, P., Children's Medical Research Institute, Sydney, Australia: Introduction to mouse development; Gastrulation/body plan.
Threadgill, D., University of North Carolina, Chapel Hill: Quantitative traits.
Trainor, P., Stowers Institute for Medical Reseach, Kansas City, Missouri: Neural crest cells and crainofacial development; Axis establishment and polarity in preimplanation embryos.
Woychik, R., The Jackson Laboratory, Bar Harbor, Maine: Mouse genetics.

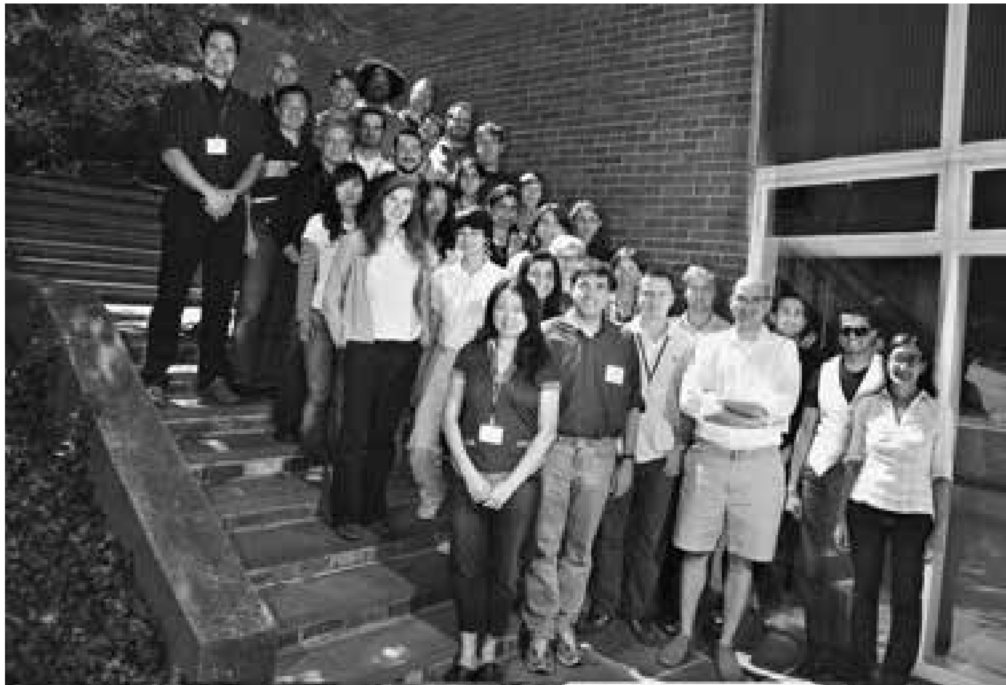
Integrative Statistical Analysis of Genome-scale Data

June 11–24

INSTRUCTORS
H. Bussemaker, Columbia University, New York
V. Carey, Harvard University, Boston, Massachusetts
C. Leslie, Memorial Sloan-Kettering Cancer Center, New York
P. Mitra, Cold Spring Harbor Laboratory
M. Reimers, Virginia Commonwealth University, Richmond

ASSISTANTS
M. Fazlollahi, Columbia University, New York
E. Lee, Columbia University, New York
J. Mar, Harvard University, Cambridge, Massachusetts
J. Zhang, Harvard University, Cambridge, Massachusetts

Availability of a variety of genome-scale data sets, and the need to integrate such data sets, is a central feature of modern biological research. Experimental and computational biologists seeking to make sense of such data sets need to have a firm grasp of the relevant statistical and analytical methodology. This course was designed to build competence in quantitative methods for the analysis of high-throughput molecular biology data. Topics included introduction to R and Bioconductor, review of multivariate statistics (multiple testing, regression, machine learning), survey of key high-throughput technologies (both microarray and sequencing based), low-level microarray data analysis (quality control, normalization), analysis based on predefined gene sets (e.g., Gene Ontology), classification and prognosis of cancer samples by machine learning, *cis*-regulatory sequence analysis (motif finding, weight matrices), modeling of transcriptional networks through integration of mRNA expression, ChIP, and sequence data, integration of genotype (SNP) data and expression data, and integration of epigenetic (DNA methylation) data and expression data.



Detailed lectures and presentations by guest speakers in the morning and evening were combined with hands-on computer tutorials in the afternoon, in which the methods covered in the lectures were applied to actual high-throughput data for yeast and humans. Students were assumed to have a basic familiarity with the R programming language at the start of the course.

Speakers this year included Stefan Bekiranov (University of Virginia, Charlottesville), Martha Bulyk (Brigham and Womens Hospital and Harvard Medical School), Bruce Futcher (Stony Brook University), Vishy Iyer (University of Texas, Austin), Robert Lucito (Cold Spring Harbor Laboratory), Trudy MacKay (North Carolina State University), Ewy Mathe (National Cancer Institute), Adam Olshen (Memorial Sloan-Cancer Center), Dana Pe'er (Columbia University), and John Stamatoyannopoulos (University of Washington).

This course is supported with funds provided by the National Cancer Institute (http://www.cancer.gov/).

PARTICIPANTS

Bernal-Mizrachi, L., M.D., Winship Cancer Institute/Emory University, Atlanta, Georgia
Beutler, A., Ph.D., Mount Sinai School of Medicine, New York
Brynedal, B., M.Sc., Karolinska Institutet, Stockholm, Sweden
Cesaroni, M., M.S., European Institute of Oncology, Milan, Italy
Crapoulet, N., Ph.D., Atlantic Cancer Research Institute, New Brunswick, Canada
Davis, A., Ph.D., Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts
Du, R., Ph.D., Harvard Medical School/Brigham and Women's Hospital, Boston, Massachusetts
Ge, B., Ph.D., McGill University, Montreal, Canada
Gruntman, E., M.Sc., Cold Spring Harbor Laboratory
Hammer, P., M.Sc., University of Applied Sciences Wildau Bioinformatics, Germany
Himes, B., Ph.D., Harvard Medical School, Boston, Massachusetts
Jakkula, L., Ph.D., Lawrence Berkeley National Laboratory, Berkeley, California
Klijn, C., M.Sc., Netherlands Cancer Institute, Amsterdam, The Netherlands
Lee, C.-K., Ph.D., Allen Institute for Brain Science, Seattle, Washington
Lu, N., M.S., Kansas State University, Manhattan
Marko, N., M.D., Cleveland Clinic, Ohio
Ottoboni, L., Ph.D., Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts
Pattyn, F., Ph.D., Ghent University, Belgium
Rabin, K., M.D., Baylor College of Medicine, Houston, Texas
Ravits, J., M.D., Benaroya Research Institute, Seattle, Washington
Shaknovich, R., M.D., Albert Einstein College of Medicine, Bronx, New York
van Wageningen, S., M.A., University Medical Centre Utrecht, The Netherlands
Weaver, D., Ph.D., University of Toledo, Ohio
Weinhold, N., M.Sc., Technical University of Denmark, Lyngby
Zavodszky, M., Ph.D., Michigan State University, E. Lansing
Zhao, X., M.S., University of Oslo/Radium Hospital, Norway

SEMINARS

Bekiranov, S., University of Virginia, Charlottesville: Intro to high-throughput sequencing.
Bulyk, M., Brigham and Womens Hospital and Harvard Medical School, Boston, Massachusetts: Protein binding.
Bussemaker, H., Columbia University, New York: Basic gene set analysis; Representations TF sequence specificity; Methods for de novo motif discovery; Transcriptional networks I; Transcriptional networks II; Posttranscriptional networks in yeast.
Futcher, B., Stony Brook University, New York
Iyer, V., University of Texas, Austin: Transcription analysis with DNA microarrays and high-throughput sequencing; ChIP-chip and ChIP-Seq.
Leslie, C., Memorial Sloan-Kettering Cancer Center, New York: Cancer classifications; MEDUSA; Regulation of mRNA stability and translation by microRNAs.
Lucito, R., Cold Spring Harbor Laboratory: Methylation assays.
MacKay, T., North Carolina State University, Raleigh: Systems genetics of complex traits in *Drosophila*.
Mitra, P., Cold Spring Harbor Laboratory: Solexa.
Olshen, A., Memorial Sloan-Kettering Cancer Center, New York: aCGH data.
Pe'er, D., Columbia University, New York: Genetic variation and regulatory networks.
Reimers, M., Virginia Commonwealth University, Richmond: Advanced gene set analysis; Multiple comparisons; Integrating epigenetic and expression data.
Snyder, M., Yale University, New Haven, Connecticut: Transcriptional networks; Proteomics.

Proteomics

June 11–24

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	**A. Farley,** Vanderbilt University, Nashville, Tennessee **F. Festa,** Harvard Medical School, Cambridge, Massachusetts **E. Hainsworth,** Harvard Medical School, Cambridge, Massachusetts **E. Simon,** University of Michigan, Ann Arbor **S. Srivastava,** Harvard University, Cambridge, Massachusetts **S. Volk,** University of Michigan, Blissfield

This intensive laboratory and lecture course focused on two major themes in proteomics: protein profiling and functional proteomics. The profiling section taught students 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. The functional proteomics section taught students 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 perform and analyze proteomics experiments and to learn to identify new opportunities in applying proteomics approaches to their own research.

Speakers in the 2008 course included Roger Biringer (Life Sciences Mass Spectrometry), Steven Carr (Broad Institute of Harvard/Massachusetts Institute of Technology), Brian Chait (The Rockefeller University), Pierre Chaurand (Vanderbilt University School of Medicine), Karl Clauser (Broad Institute of Harvard/Massachusetts Institute of Technology), Illeana Cristea (Princeton University), Paul Huang (Massachusetts Institute of Technology), Darryl Pappin (Cold Spring Harbor Laboratory), and Michael Snyder (Yale University).

This course was supported with funds provided by the National Cancer Institute (http://www.cancer.gov/).

PARTICIPANTS

Adams, S., Ph.D., Mayo Clinic Jacksonville, Florida
Bokowiec, M., M.A., University of Virginia, Charlottesville
Cabrejos, M.E., Ph.D., University of Chile, Santiago
Charles, R., M.D., Massachusetts General Hospital, Boston
Chuang, A., M.S., University of Iowa, Iowa City
Conlon, F., Ph.D., University of North Carolina, Chapel Hill
Eriksson, C., M.S., Royal Institute of Technology, Stockholm, Sweden
Mahajan, V., Ph.D., University of Iowa, Iowa City
Moradian, A., Ph.D., Michael Smith Genome Sciences Centre, Vancouver, Canada
Padula, M., B.S., Albert Einstein College of Medicine, Bronx, New York
Ramos-Ortolaza, D., B.S., Mount Sinai School of Medicine, New York
Rechavi, O., Ph.D., Tel Aviv University, Israel
Shaughnessy, D., Ph.D., NIEHS-DERT, Research Triangle Park, North Carolina
Skaug, B., B.S., University of Texas Southwestern Medical Center, Dallas
Soufi, B., B.Sc., Technical University of Denmark, Lyngby
Wisniewski, P., M.S., The Nencki Institute of Experimental Biology, Warsaw, Poland

SEMINARS

Carr, S., Broad Institute of Harvard/Massachusetts Institute of Technology, Cambridge: Mass spec pipelines for biomarker discovery.
Chait, B., The Rockefeller University, New York: Protein interactions as a window into cellular function.
Chaurand, P., Vanderbilt University School of Medicine, Nashville, Tennessee: Profiling tissues by mass spectrometry.
Clauser, K., Broad Institute/Massachusetts Institute of Technology, Cambridge: De novo interpretation of tandem mass spectra.
Cristea, I., The Rockefeller University, New York: Protein interactions.
Huang, P., Massachusetts Institute of Technology, Charlestown: Phosphoproteomics.
Synder, M., Yale University, New Haven, Connecticut: Application of protein microarrays.

Computational Neuroscience: Vision

June 20–July 3

INSTRUCTORS	**G. Boynton,** University of Washington, Seattle **G. Horwitz,** University of Washington, Seattle **S. Treue,** German Primate Center, Goettingen, Germany
ASSISTANTS	**G. Field,** Salk Institute, La Jolla, California **S. Hohl,** University of California, San Francisco
TECHNICIAN	**R. Dotson,** New York University, New York

Computational approaches to neuroscience will produce important advances in our understanding of neural processing. Prominent success will come 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 can help to 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 in this year's course included Geoffrey Boynton, David Brainard, Matteo Carandini, Marisa Carrasco, E.J. Chichilnisky, Wilson Geisler, Joshua Gold, David Heeger, Gregory Horwitz, Bart Krekelberg, Partha Mitra, Tony Movshon, Andrew Parker, Anitha Pasupathy, Pamela Reinagel, Eero Simoncelli, Alex Thiele, Frank Tong, and Stefan Treue.

The course was held at the Laboratory's Banbury Conference Center (http://www.cshl.edu/banbury/) 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

Ayaz, A., B.S., University of California, Irvine
Brouwer, G.J., Ph.D., New York University, New York
Cohen, J., B.S., Vanderbilt University, Nashville, Tennessee
Daliri, M., Ph.D., German Primate Center, Goettingen, Germany
Doi, T., B.S., Osaka University, Toyonaka, Japan
Elstrott, J., B.S., University of California, San Diego
Fitzgerald, J., B.S., Harvard Medical School, Boston, Massachusetts
Girshick, A., Ph.D., New York University, New York
Hartmann, T., B.S., Rutgers University, Newark, New Jersey
Karklin, Y., B.S., New York University, New York
Kerlin, A., B.S., Harvard Medical School, Boston, Massachusetts
Kherlopian, A., B.S., Weill Cornell Medical College, New York
Koster, U., B.S., University of Helsinki, Finland
Law, C.-T., B.S., University of Pennsylvania, Philadelphia
Manookin, M., B.S., University of Michigan, Ann Arbor
Michel, M., Ph.D., University of Texas, Austin
Muhammad, R., B.S., Massachusetts Institute of Technology, Cambridge
Murphy, D., B.S., Baylor College of Medicine, Houston, Texas
Pestilli, F., B.S., New York University, New York
Rutledge, R., B.S., New York University, New York
Schmid, M., Ph.D., National Institutes of Mental Health, Bethesda, Maryland
Sharan, L., B.S., Massachusetts Institute of Technology, Cambridge
Tavassoli, A., B.S., University of California, Los Angeles
Wallisch, P., Ph.D., New York University, New York

SEMINARS

Brainard, D., University of Pennsylvania, Philadelphia: Trichromacy.
Carandini, M., Smith-Kettlewell Eye Research Institute, San Francisco, California: Adaptation/LGN.
Carrasco, M., New York University, New York: Attention psychophysics.
Chichilnisky, E.J., The Salk Institute, La Jolla, California: Visual procesing in the retina; Retinal population codes.
Geisler, B., University of Texas, Austin: Pattern vision and natural scenes.
Gold, J., University of Pennsylvania, Philadelphia: Decisions, choice, and neuroeconomics.
Heeger, D., New York University, New York: V1 physiology/models; Organization of extrastriate visual cortex.
Horwitz, G., University of Washington, Seattle: V1/white noise analysis.
Krekelberg, B., Rutgers University, Newark, New Jersey: Population coding/decoding.
Mitra, P., Cold Spring Harbor Laboratory: Observed brain dynamics.
Movshon, T., New York University, New York: What vision (and this course) is all about.
Parker, A., Oxford University, United Kingdom: Stereo vision: Physiology.
Pasupathy, A., University of Washington, Seattle: Ventral stream, form vision.
Reinagel, P., University of California, San Diego, La Jolla: LGN coding and natural scene statistics.
Simoncelli, E., New York University, New York: Motion modeling; Image statistics.
Thiele, A., Newcastle University, Newcastle Upon Tyne, United Kingdom: MT physiology.
Tong, F., Vanderbilt University, Nashville, Tennessee: fMRI: Advanced methods.
Treue, S., German Primate Center, Goettingen, Germany: Attention physiology.

Advanced Techniques in Molecular Neuroscience

June 27–July 13

INSTRUCTORS

J. Eberwine, University of Pennsylvania Medical School, Philadelphia
J. Kohler, Stanford University, California
C. Lai, Scripps Research Institute, La Jolla, California
R. Lansford, California Institute of Technology, Pasadena

ASSISTANTS

T. Bell, University of Pennsylvania, Philadelphia
P. Buckley, University of Pennsylvania, Philadelphia
C. Challis, Scripps Research Institute, La Jolla, California
D. Huss, California Institute of Technology, Covina, California
G. Poynter, California Institute of Technology, Pasadena, California
K. Schaukowitch, Scripps Research Institute, La Jolla, California

This newly revised laboratory and lecture course was designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the most advanced techniques in molecular neuroscience. The course curriculum was divided into three sections: an extensive and up-to-date set of laboratory exercises, daily lectures covering the theoretical and practical aspects of the various methods used in the laboratory, and a series of evening research seminars. The informal and interactive evening lectures were given by leading molecular neuroscientists and serve 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 (siRNAs) 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 available to molecular neuroscientists.

Speakers in the 2008 course included Cori Bargmann (The Rockefeller University), Ben Barres (Stanford University School of Medicine), Karl Deisseroth (Stanford University), Kenneth Kosik (Neuroscience Research Institute), Jeff Lichtman (Harvard University), Pierre-Marie Lledo (Pasteur Institute), Donald Lo (Duke Center for Drug Discovery), Joseph Loturco (University of Connecticut), Ardem Patapoutian (The Scripps Reseach Institute), and Eduardo Rosa-Molinar (University of Puerto Rico-Rio Piedras).

This course is supported with funds provided by the National Institute of Mental Health (http://www.nimh.nih.gov/), the National Institute of Neurological Disorders & Stroke (http://www.ninds.nih.gov/), and the Howard Hughes Medical Institute (http://www.hhmi.org/). Scientists from developing countries accepted into this course may be eligible for scholarships provided by the International Brain Research Organization (http://www.iac-usnc.org/fellowships.html).

PARTICIPANTS

Adams, S., Ph.D., Mayo Clinic Jacksonville, Florida
Corty, M., B.A., Columbia University, New York
Dagostin, A.L., University of São Paulo, Ribeirão Preto, Brazil
Djurisic, M., Ph.D., Stanford University, California
Edgar, N., M.S., University of Pittsburgh, Pennsylvania
Garcia-Pena, C., B.S., UNAM, Instituto de Neurobiologia, Juriquilla, Mexico
Karlen, S., B.S., University of California, Davis
Kim, Y., Ph.D., University of Oxford, United Kingdom
Ludanyi, A., M.Sc., Institute of Experimental Medicine, Budapest, Hungary
Luo, M., Ph.D., National Institute of Biological Sciences, Beijing, China
Maya Vetencour, J., M.S., Scuola Normale Superiore, Pisa, Italy
Nimmerjahn, A., Stanford University, California
Pathak, A., M.S., SG Postgraduate Institute of Medical Sciences, Lucknow, India
Vogelstein, J., B.S., Johns Hopkins University, Baltimore, Maryland
Wozny, C., M.D., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
Zhou, C., B.S., National Institute of Biological Sciences, Beijing, China

SEMINARS

Bargmann, C., The Rockefeller University, New York: Mixer with FlyCourse.
Barres, B., Stanford University School of Medicine, California: Glial cell development.
Deisseroth, K., Stanford University, California: Optogenetics module.
Eberwine, J., University of Pennsylvania Medical School, Philadelphia: RNA isolation from single cells.
Haas, K., University of British Columbia, Canada: *Xenopus* neural transfection.
Kosik, K., Neuroscience Research Institute, Santa Barbara, California: MicroRNAs in neural systems.
Lai, C., Scripps Research Institute, La Jolla, California: BAC recombination module.
Lansford, R., California Institute of Technology, Pasadena: Cloning and lentivirus module.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Analysis of “Rainbow” mice.
Lledo, P.-M., Pasteur Institute, Paris, France: Becoming an adult olfactory neuron.
Lo, D., Duke University Medical Center, Durham, North Carolina: BAC transfections.
Loturco, J., University of Connecticut, Storrs: RNAi module.
Patapoutian, A., Scripps Research Institute, La Jolla, California: The fifth sense: Molecular mechanisms of temperature and pain detection.

Computational Cell Biology

June 27–July 17

INSTRUCTORS	**T. Elston,** University of North Carolina, Chapel Hill **C. Fall,** University of Illinois, Chicago **L. Loew,** University of Connecticut Health Center, Farmington **G. Smith,** College of William and Mary, Williamsburg, Virginia **J. Tyson,** Virginia Polytechnic Institute and State University, Blacksburg
CO-INSTRUCTORS	**J. Hasty,** University of California, San Diego, La Jolla **A. Mogilner,** University of California, Davis **J. Sible,** Virginia Polytechnic Institute and State University, Blacksburg
ASSISTANTS	**D. Barik,** Virginia Polytechnic Institute and State University, Blacksburg **B. Baumgartner,** University of California, San Diego, La Jolla **P. Kraikivski,** University of Connecticut Health Center, Farmington

Computational cell biology is the field of study that applies the mathematics of dynamical systems together with computer simulation techniques to the study of cellular processes. The field encompasses several topics that have been studied long enough to be well established in their own right, such as calcium signaling, molecular motors, and cell motility; the cell cycle; and gene expression during development. In addition to providing a recognizable larger community for topics such as these, this course provided a base for the development of newer areas of inquiry, for example, the dynamics of intracellular second-messenger signaling, programmed cell death, mitotic chromosome movements, and synthetic gene networks. Unlike computational genomics or bioinformatics, computational cell biology is focused on simulation of the molecular machinery (genes–proteins–metabolites) that underlie the physiological behavior (input–output characteristics) of living cells.

This 3-week course incorporated a series of didactic lectures on the mathematics of dynamical systems, computational simulation techniques, cell biology, and molecular biology. Practicing theoreticians and experimentalists rotated in for 1–3-day visits during the course to give lectures and interact with the students.

Midway through the course, students selected an area for independent study, and the focus of the last week of the course was largely on these projects, supplemented by continued visiting lecturers: Reka Albert (Pennsylvania State University), Dean Bottino (Novartis), Barbara Ehrlich (Yale University School of Medicine), Jason Haugh (North Carolina State University), Ravi Iyengar (Mount Sinai School of Medicine), James Keener (University of Utah), Edda Klipp (Humboldt University and Max-Planck Institute), James Lechleiter (University of Texas Heath Sciences Center), Jennifer Linderman (University of Michigan), Charles Peskin (New York University), Artie Sherman (National Institutes of Health/NIDDK), Stas Shvartsman (Princeton University), Boris Slepchenko (University of Connecticut Health Center), and David Terman (Ohio State University).

Visiting faculty will change from year to year, and the specific topics covered will vary. Potential areas include the Fundamentals (e.g., cell biology, molecular biology, mathematical biology, and computational tools), advanced topics (e.g., sniffers, buzzers, toggles, and blinkers; practical bifurcation theory; reaction-diffusion-advection; stochastic modeling; physical chemistry of aggregation/polymerization; mechano-chemical dynamics; sensitivity and robustness; optimization, parameter estimation); and case studies (calcium signaling; cancer and cardiac modeling; signal transduction networks; gene expression; apoptosis; cell cycle regulation; cytoskeletal dynamics; and neural models).

This course was supported with funds provided by the National Science Foundation, Apple Computer (computer support), and Mathworks (MATLAB licenses).

PARTICIPANTS

Brakken-Thai, C., B.A., University of Minnesota, Minneapolis
Carpenter, R., B.S., College of William and Mary, Williamsburg, Virginia
Coxito, P., B.S., Centre for Neuroscience and Cell Biology, Coimbra, Portugal
de Diego, A., Ph.D., Universidad Autonoma, Madrid, Spain
Durrieu, L., M.Sc., IFIByNE, CONICET
Elsner, M., Ph.D., National Institutes of Health, NICHD, Bethesda, Maryland
Farre, E., Ph.D., University of California, San Diego, La Jolla
Gonzales, P., B.S., National Institutes of Health/NHLBI, Bethesda, Maryland
Hao, Y., B.S., College of William and Mary, Williamsburg, Virginia
Hoyos Ramirez, E., B.A., Ingenieur d'Etude, Paris, France
Osterfield, M., Ph.D., Harvard Medical School, Boston, Massachusetts
Picone, R., B.Sc., University College London, United Kingdom
Sanz-Rodriguez, C., B.S., Universidad Simon Bolivar, Caracus, Venezuela
Schwemmer, M., B.A., University of California, Davis
Waks, Z., B.S., Harvard Medical School, Boston, Massachusetts:
Wong, M., B.S., University of California, San Diego
Zamborszky, J., M.S., University of Trento CoSBi, Italy

SEMINARS

Albert, R., Pennsylvania State University, University Park: Discrete dynamic modeling of signaling networks.
Bottino, D., Novartis Pharmaceuticals Corporation, East Hanover, New Jersey: Pharma/cardio toxicity; Pharma/cancer modeling.
Ehrlich, B., Yale University, New Haven, Connecticut: IP3 receptors and Ca^{2+} signaling.
Haugh, J., North Carolina State University, Raleigh: Kinetic and spatial analysis of signal transduction pathways and networks.
Iyengar, R., Mount Sinai School of Medicine, New York: Network building and tracking new pathways; Spatial regulation of information flow in cells.
Keener, J., University of Utah, Salt Lake City: Cardiac models.
Lechleiter, J., University of Texas, San Antonio: Biology of calcium signaling.
Linderman, J., University of Michigan, Flint: Modeling G-protein-coupled receptors.
Loew, L., University of Connecticut, Storrs: Introduction to Ca^{2+} models; Dendritic nucleation models.
Mogilner, A., University of California, Davis: Stochastic models and techniques; Force balance between antagonistic molecular motors; Stochastic, yet faithful, search-and-capture mechanism in mitotic spindle assembly.
Sherman, A., National Institutes of Health, Bethesda, Maryland: Integrated Ca^{2+} models.
Sible, J., Virginia Polytechnic Institute, Blacksburg, Virginia: Biology of cell signaling.
Slepchenko, B., University of Connecticut, Storrs: Membranes and ion channels: Modeling approaches in electrophysiology.
Smith, G., College of William and Mary, Williamsburg, Virginia: Cellular biophysics and ODEs; Reaction-diffusion and advection; Markov chain models of ion channels and calcium release sites.
Terman, D., Ohio State University Mathematical Biosciences Institute, Columbus: ODEs: Bifurcation continuation; Mathematics of waves in biology.
Tyson, J., Virginia Polytechnic Institute, Blacksburg: Models of MPF regulation; Temporal organization of the cell cycle.

Molecular Techniques in Plant Science

June 27–July 17

INSTRUCTORS **T. Brutnell,** Boyce Thompson Institute, Ithaca, New York
V. Irish, Yale University, New Haven, Connecticut
E. Kellogg, University of Missouri, St. Louis
J. Normanly, University of Massachusetts, Amherst

ASSISTANTS **J. Jung,** Cornell University, Ithaca, New York
B. Kokturk, University of Massachusetts, Amherst
R. Reinheimer, University of Missouri, St. Louis

This course provided an intensive overview of topics in plant physiology, biochemistry, and development, focusing on molecular genetic and analytical approaches to understanding plant biology. It emphasized recent results from *Arabidopsis*, maize, and a variety of other plants including *Brachypodium nicotiana* 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 plants using the latest technologies in genetics, molecular biology, and biochemistry. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Discussions of important topics in plant research were presented by the instructors and by invited speakers. These seminars included plant morphology and anatomy; plant development (such as development of flowers, leaves, male and female gametophytes, and roots); perception of light and photomorphogenesis; cell wall biosynthesis; function and perception of hormones; and application of research results to addressing current agronomic problems including the rapidly advancing field of cellulosic biofuel production and feedstock development. Speakers provided overviews of their fields, fol-

lowed by in-depth discussions of their own work. The laboratory sessions provided an introduction to important techniques currently used in plant research. These included the application of light, fluorescence, and scanning electron microscopy to the study of plant development; mutant analysis; transient gene expression; gene silencing; applications of fluorescent protein fusions; protein interaction and detection; proteomics approaches; several different approaches to quantifying metabolites; and transient transformation and techniques commonly used in genetic and physical mapping. The course also included several short workshops on important themes in plant research.

Speakers in the course included Thomas Brutnell (Boyce Thompson Institute), John Celenza (Boston University), Savithramma Dinesh-Kumar (Yale University), Kevin Folta (University of Florida), Erich Grotewold (Ohio State University), Stacey Harmer (University of California, Davis), Vivian Irish (Yale University), Dave Jackson (Cold Spring Harbor Laboratory), Georg Jander (Boyce Thompson Institute), Elizabeth "Toby" Kellogg (University of Missouri, St. Louis), Nancy Kerk (Yale University), Cris Kuhlemeier (University of Bern), Jennifer Normanly (University of Massachusetts), Thomas Nuhse (University of Manchester), Jocelyn Rose (Cornell University), Eric Schaller (Dartmouth College), Ian Sussex (Yale University), and Marja Timmermans (Cold Spring Harbor Laboratory). This course was supported with funds provided by the National Science Foundation

PARTICIPANTS

Butler, H., M.S., Dow AgroSciences, Indianapolis, Indiana
Chang, P.-C., M.S., University of Waterloo, Canada
Danielson, J., M.Sc., Lund University, Sweden
Hendrickson Culler, A., Ph.D., University of Minnesota, St. Paul
Hepworth, J., B.A., University of York, United Kingdom
Matoba, N., Ph.D., Arizona State University, Tempe
Roos, J., M.Sc., Swedish University of Agricultural Sciences, Uppsala, Sweden
Sønderby, I., B.A., University of Copenhagen, Denmark
Sørmo, C., M.S., Norwegian University of Science and Technology, Trondheim, Norway
Stenbaek, A., M.S., University of Copenhagen, Denmark
Whitcomb, S., B.A., The Rockefeller University, New York
Worden, S., B.S., Dow AgroSciences, Indianapolis, Indiana

SEMINARS

Benfey, P., Duke University, Durham, North Carolina: Root development.
Brutnell, T., Boyce Thompson Institute, Ithaca, New York; Irish, V., Yale University, New Haven, Connecticut: Workshop I: Double mutants, genetic maps; Workshop III: Solexa sequencing.
Brutnell, T., Boyce Thompson Institute, Ithaca, New York: Plastids.
Celenza, J., Boston University, Massachusetts: Secondary metabolites.
Dinesh-Kumar, S., Yale University, New Haven, Connecticut: Plant pathogen interactions.
Folta, K., University of Florida, Gainesville: Light regulation.
Grotewold, E., University of Ohio, Columbus: Regulatory networks.
Harmer, S., University of California, Davis: Circadian rhythms.
Irish, V., Yale University, New Haven, Connecticut: Reproductive development.
Jackson, D., Cold Spring Harbor Laboratory: Shoot meristem development.
Jander, G., Boyce Thompson Institute, Ithaca, New York: Quantitative genetics.
Kellogg, E., University of Missouri, St. Louis: Phylogenetics.
Kuhlemeier, C., University of Bern, Switzerland: Phyllotaxis.
Normanly, J., University of Massachusetts, Amherst: Workshop III: Scientific writing.
Nuhse, T., University of Manchester, United Kingdom: Proteomics.
Rose, J., Cornell University, Ithaca, New York: Cell walls.
Schaller, G.E., Dartmouth College, Hanover, New Hampshire: Plant hormones.
Sheen, J., Massachusetts General Hospital, Boston: Signaling.
Sussex, I., Yale University, New Haven, Connecticut: Introduction to plant structure.
Timmermans, M., Cold Spring Harbor Laboratory: MicroRNA regulation.
Topp, C., University of Georgia, Atlanta
Vanzulli, S., University of Milan, Italy
Urbanowicz, B., Cornell University, Ithaca, New York: Biofuels data analysis workshop
Vogel, J., Western Regional Research Center (USDA-ARS): Feedstock development for biofuels.
Xie, Z., Ohio State University, Columbus: ChIP-chip data analysis workshop.

Neurobiology of *Drosophila*

June 27–July 17

INSTRUCTORS

R. Allada, Northwestern University, Evanston, Illinois
M. Freeman, University of Massachusetts Medical School, Worcester
B. Zhang, University of Oklahoma, Norman

ASSISTANTS

H. Bao, University of Oklahoma, Norman
R. Bernardos, University of Massachusetts Medical School, Worcester
R. Islam, University of Oklahoma, Norman
C. Pfeiffenberger, Northwestern University, Evanston, Illinois

This laboratory/lecture course is intended for researchers at all levels, from beginning graduate students through established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The 3-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that are used in the investigation of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetics approaches available in *Drosophila*, students learned a variety of techniques including embryo in situ hybridization, labeling of identified neurons, electrophysiology (intracellular recording and patch clamping), optical imaging of neuronal activity, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system.

This year's lecturers included Cori Bargmann (The Rockefeller University), Richard Benton (University of Lausanne), Heather Broihier (Case Western University), Vivian Budnik (University of Massachusetts), John Carlson (Yale University), Sarah Certel (Harvard Medical School), Yick-Bun Chan (Harvard Medical School), David Deitcher (Cornell University), Josh Dubnau (Cold Spring Harbor Laboratory), Aki Ejiima (Brandeis University), Tanja Godenschwege (Florida Atlantic University), Stephen Goodwin (University of Glasgow), Wesley Greuber (Columbia University), Randall Hewes (University of Oklahoma), Gregory Jefferis (University of Cambridge), Haig Keshishian (Yale University), Edward Kravitz (Harvard Medical School), Cheng-Yu Lee (University of Michigan, Life Sciences Institute), Edwin Levitan (University of Pittsburgh), Jill Penn (Harvard Medical School), Adrian Rothenfluh (University of California, San Francisco), Stephan Sigrist (University of Wurzburg), Sean Speese (University of Massachusetts), Glenn Turner (Cold Spring Harbor Laboratory), Troy Zars (University of Missouri), J. Lynn Zimmerman (University of Maryland Baltimore County), and John Zimmerman (University of Pennsylvania).

This course was supported with funds provided by the National Institute of Mental Health (http://www.nimh.nih.gov/), the National Institute of Neurological Disorders & Stroke (http://www.ninds.nih.gov/), and the Howard Hughes Medical Institute (http://www.hhmi.org/).

Scientists from developing countries accepted into this course may be eligible for scholarships provided by the International Brain Research Organization. For details, go to http://www.iac-usnc.org/fellowships.html).

PARTICIPANTS

Caron, S., B.S., Columbia University, New York
Chen, Y.-C., B.S., University of North Carolina, Chapel Hill
Dias, B., M.S., University of Texas, Austin
Gu, T., University of Oklahoma, Norman
Hsu, Y.-W., University of Washington, Seattle
Jaiswal, M., M.Sc., Baylor College of Medicine, Houston, Texas
Jorchel, S., M.S., Vienna Campus Biocenter, Austria
Kain, J., B.A., Harvard University, Cambridge, Massachusetts
Mendes, C., B.S., Columbia University, New York
Ostrovsky, A., B.S., University of Arizona, Phoenix
Rathore, K., M.S., National Centre for Biological Sciences, TIFR, Bangalore, India
Sakurai, A., B.S., Tohoku University, Sendai, Japan

SEMINARS

Allada, R., Northwestern University, Evanston, Illinois: Introduction; Circadian rhythms and sleep.
Bargmann, C., The Rockefeller University, New York
Benton, R., University of Lausanne, Switzerland: Odorant decoding by single sensilla.
Broihier, H., Case Western University, Cleveland, Ohio: Generation of neural diversity.
Budnik, V. and Speese, S., University of Massachusetts Medical School, Worcester: Development of the NMJ.
Carlson, J., Yale University, New Haven, Connecticut: Larval behavior.
Deitcher, D., Cornell University, Ithaca: SNAREs and transmitter release.
Featherstone, D., University of Illinois, Chicago: Development and regulation of glutamatergic signaling.
Freeman, M., University of Massachusetts Medical School, Worcester: Fly genetics 101; Glial cell biology; Fly web tools/informatics 101.
Godenschwege, T., Florida Atlantic University, Boca Raton: No escape: The giant fiber system.
Goodwin, S., Glasgow University, United Kingdom: Control of sexual behavior in *Drosophila* by the sex determination pathway.
Ejima, A., Brandeis University, Waltham, Massachusetts: Courtship and learning.
Grueber, W., Columbia University, New York: Dendrite biology.
Hewes, R., University of Oklahoma, Norman: Neuropeptide signaling.
Jefferis, G., University of Cambridge, United Kingdom: Genetic mosaic techniques; Olfactory system development.
Keshishian, H., Yale University, New Haven, Connecticut: Neuronal activity and synaptic plasticity.
Kravitz, E. and Certel, S., Harvard Medical School, Boston, Massachusetts: Aggression.
Lee, C.-Y., University of Michigan, Ann Arbor: Neural stem cells.
Levitan, E., University of Pittsburgh, Pennsylvania: Optical imaging of neuropeptide release.
Rothenfluh, A., University of Texas Southwestern, Dallas: Flies and drugs.
Sigrist, S., University of Wurzburg, Germany: Shedding light on assembly of synapse structure and function.
Turner, G., Cold Spring Harbor Laboratory: Electrophysiology 102; Neural coding in the fly brain: In vivo whole-cell patch-clamp recording methods.
Zars, T., University of Missouri, Columbia and Dubnau, J., Cold Spring Harbor Laboratory: Olfactory learning and memory.
Zhang, B., University of Oklahoma, Norman: Electrophysiology 101; The synaptic vesicle cycle.

Revolutionary Sequencing Technologies and Applications

July 6–17

INSTRUCTORS

E. Mardis, Washington University School of Medicine, St. Louis, Missouri
G. Marth, Boston College, Chestnut Hill, Massachusetts
R. McCombie, Cold Spring Harbor Laboratory
J. McPherson, Ontario Institute for Cancer Research, Toronto, Canada
M. Zody, Broad Institute, Cambridge, Massachusetts

ASSISTANTS

M. Busby, Boston College, Chestnut Hill, Massachusetts
J. Glasscock, Washington University School of Medicine, St. Louis, Missouri
E. Hodges, Cold Spring Harbor Laboratory
M. Kramer, Cold Spring Harbor Laboratory
V. Magrini, Washington University School of Medicine, St. Louis, Missouri
M. Stromberg, Boston College, Chestnut Hill, Massachusetts

During the last decade, large-scale DNA sequencing has had a marked impact on the practice of modern biology and is beginning to affect the practice of medicine. With the recent introduction of several revolutionary sequencing technologies, costs and time lines have been reduced by orders of magnitude, facilitating investigators to conceptualize and perform sequencing-based projects that heretofore were prohibitive. Furthermore, the application of these technologies to answer questions previously not experimentally approachable is broadening their impact and application.

This intensive 12-day course explored applications of next-generation sequencing technologies, with a focus on commercially available methods. Students were instructed in the detailed operation of several revolutionary sequencing platforms, including sample preparation procedures, general data

handling through pipelines, and in-depth data analysis. A diverse range of biological questions were explored including DNA resequencing of human genomic regions (using cancer samples as a test case), de novo DNA sequencing of bacterial genomes, and the use of these technologies in studying small RNAs, among others. Guest lecturers highlighted their own applications of these revolutionary technologies.

We encouraged applicants from a diversity of scientific backgrounds, including molecular evolution, development, neuroscience, cancer, plant biology, and microbiology.

This course was sponsored by Applied Biosystems, Illumina, and 454 Life Sciences (http://www.454.com/).

PARTICIPANTS

Beutler, A., Ph.D., Mount Sinai School of Medicine, New York
Cattonaro, F., Ph.D., Institute of Applied Genomics, Udine, Italy
Chen, N., B.A., Cornell University, Ithaca, New York
Cho, M., Channing Laboratory, Brigham and Women's Hospital, Boston, Massachusetts
Elshire, R., M.S., Cornell University, Ithaca, New York
Goes, F., M.D., Johns Hopkins University, Baltimore, Maryland
Jafari, N., Doctorate, Northwestern University, Chicago, Illinois
Kenny, E., Trinity College Dublin, Republic of Ireland
Khan, A., M.S., AgResearch Ltd., Dunedin, New Zealand
Kieleczawa, J., M.S., Wyeth Research, Cambridge, Massachusetts
Kilalainen, A., M.Sc., Uppsala University, Finland
Kim, D., Ph.D., Beckman Research Institute of the City of Hope, Duarte, California
Kinney, J., B.A., Princeton University, New Jersey
Kuenzli, M., Functional Genomics Center, Zurich, Switzerland
Pelizzola, M., Ph.D., Yale University, New Haven, Connecticut
Rasmussen, M., M.S., University of Copenhagen, Denmark
Rose, M., Ph.D., Syngenta Biotechnology, Inc., Research Triangle Park, North Carolina
Sengamalay, N., M.S., Institute for Genomic Sciences, Baltimore, Maryland
Waterfall, J., Ph.D., Cornell University, Ithaca, New York

SEMINARS

Aravin, A., Cold Spring Harbor Laboratory: Discovering small RNAs with Solexa sequencing.
Bloom, T., Broad Institute of MIT and Harvard University, Cambridge, Massachusetts: Infrastructure and IT for next-generation sequencing.
Egholm, M., 454 Life Sciences/Roche, Branford, Connecticut: Future improvements to Roche/454 pyrosequencing.
Erlich, Y., Cold Spring Harbor Laboratory: Alta-cyclic: A self-optimized Illumina base-caller.
Mardis, E., Washington University School of Medicine, St. Louis, Missouri: Next-generation sequencing and cancer genomics.
Meissner, A., Broad Institute, Cambridge, Massachusetts: Dissecting direct reprogramming through integrative genomic analysis.
Olson, J. and Becker S., Raindance Technologies, Lexington, Massachusetts: Selective genomic amplification using droplet-based microfluidics.
Schroth, G., Illumina, Inc., Hayward, California: Future improvements to the Illumina Genome Analyzer.
Snyder, M., Yale University, New Haven, Connecticut: Next-generation-based RNA-seq studies of the yeast *Saccharomyces cerevisiae*.
Turner, S., Pacific Biosciences, Menlo Park, California: SMaRT sequencing.
Watson, J., Cold Spring Harbor Laboratory: Human genome.

Genetics of Complex Human Diseases

July 7–13

INSTRUCTORS **A. Al-Chalabi,** 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 and their interactions with one another and with the environment. This lecture course considered the difficulties in studying the genetic basis of complex disorders such as diabetes, cardiovascular disease, cancer, and autism. We discussed genetic-epidemiologic study designs, including family, parent-child trio, case/control and adoption studies, and 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 analysis and their variants. We discussed the efficiency and robustness of different designs for such analysis and the appropriate contexts for their use. 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. Copy-number variation as an influence on complex disease risk and Bayesian coalescent models for the fine mapping of disease loci were also discussed. Illustrations were provided through discussion of results from ongoing studies of a variety complex diseases and related risk factors.

Speakers in the course included Ingrid Borecki (Washington University), Paul De Bakker (Massachusetts General Hospital), Toby Johnson (University of Edinburgh, United Kingdom), Marina Kennerson (ANZAC Research Institute, New South Wales, Australia), Adrian Krainer (Cold Spring

Harbor Laboratory), Eden Martin (University of Miami), Shaun Purcell (Kings College London, United Kingdom), Jonathan Sebat (Cold Spring Harbor Laboratory), Pak Sham (University of Hong Kong, China), and Janet Sinsheimer (University of California, Los Angeles).

PARTICIPANTS

Asselta, R., Ph.D., University of Milan, Italy
Avenevoli, S., Ph.D., National Institute of Mental Health, Bethesda Maryland
Bione, S., Ph.D., Institute of Molecular Genetics, Pavia, Italy
Creed Geraghty, J., B.S., Weill Cornell Medical College Qatar Doha, Education City
Cutler, G., Ph.D., Amgen Informatics, South San Francisco, California
Davis, A., Ph.D., Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts
Davis, L., B.S., University of Iowa, Iowa City
Didriksen, M., Ph.D., H. Lundbeck A/S, Valby, Denmark
Ernst, F., Ph.D., University Hospital of Greifswald, Germany
Lopez, S., Ph.D., Institut de Recerca de l'Hospital de la Santa Creu, Barcelona, Spain
Macedo Vranas, M., B.S., University of Aarhus, Denmark
Manuel Soria, J., Ph.D., Institut de Recerca de l'Hospital de la Santa Creu, Barcelona, Spain
McKay, G., Ph.D., Queen's University Belfast, Belfast, United Kingdom
Mocan, E., B.S., Institute of Genetics, Chisinau, Moldava
Saito-Loftus, Y., Ph.D., Mayo Clinic, Rochester, Minnesota
Teumer, A., B.S., University of Greifswald, University Hospital, Germany
Wiechec, E., B.S., University of Aarhus, Denmark
Yeo Han, D., B.S., University of Auckland, New Zealand
Zedler, B., Ph.D., Philip Morris USA, Richmond, Virginia

SEMINARS

Almasy, L., Southwest Foundation for Biomedical Research, San Antonio, Texas: Advanced linkage.
Al-Chalabi, A., Kings College London, United Kingdom: Introduction to associational studies.
Borecki, I., Washington University School of Medicine, St. Louis, Missouri: Genetic epidemiology.
De Bakker, P., Brigham and Women's Hospital, Boston, Massachusetts: GWAS meta analysis.
Johnson, T., University of Lausanne, Vaud, Switzerland: Evolution and population genetics.
Kennerson, M., ANZAC Research Institute, New South Wales, Australia: Introduction to linkage; Genetics linkage programs practical.
Krainer, A., Cold Spring Harbor Laboratory: RNA in complex disease.
Martin, E., University of Miami, Florida: Family-based association.
Neale, B., Massachusetts General Hospital, Boston: SNP imputation.
Purcell, S., Massachusetts General Hospital, Boston: Genome-wide association studies.
Sebat, J., Cold Spring Harbor Laboratory: Copy-number variation.
Sham, P.C., University of Hong Kong, China: Association studies: Power and multiple testing issues.
Sinsheimer, J., David Geffen School of Medicine, Los Angeles, California: Statistics 101.

Workshop on Biology of Social Cognition

July 14–20

INSTRUCTORS **R. Adolphs,** California Institute of Technology, Pasadena
D. Skuse, University College London, United Kingdom

ASSISTANT **A. Lin,** California Institute of Technology, Pasadena

The past few years have seen remarkable advances in our knowledge of the genetic, molecular, and neural factors that contribute to social behavior. At the same time, sophisticated analytical and theoretical approaches have helped to make sense of the data. This week-long workshop provided a comprehensive overview of these topics. Although the emphasis was on social cognition in humans, there were study days dedicated to state-of-the-art presentations on comparative approaches and evolutionary models. Finally, all themes were related to the clinical consequences of dysfunctional social cognition and the role of translational research. The course included introductory seminars on key themes, offered every morning. Afternoons were devoted largely to practical sessions that provided hands-on experiments in consultation with seminar leaders, rounded off by after-dinner keynote lectures by leading scientists that reflected cutting-edge and future views related to our theme for that day. Ample breaks allowed time for informal interactions between lecturers and students.

PARTICIPANTS

Bodley Scott, S., B.S., University of Nottingham, United Kingdom
Campbell-Meiklejohn, D., Ph.D., University of Aarhus, Denmark
Canadas, E., B.S., University of Granada, Spain
Duman, E., B.S., Biopsychology Ph.D. Program, Stony Brook University, New York
Florentini, C., B.S., University of Geneva, Switzerland
Gao, X., Ph.D., McMaster University, Hamilton, Canada
Glaser, B., B.S., University of Geneva, Switzerland
Gupta, R., B.S., University of Iowa, Iowa City
Jones, E., B.S., University of Washington, Seattle
Lampis, V., Ph.D., San Raffaele University, Milan, Italy
Lim P., B.S., National University of Singapore
Modi, M., B.S., Emory University, Atlanta, Georgia
Oswald, T., B.S., University of Oregon, Eugene
Payne, C., B.S., Emory University/Yerkes NPRC, Atlanta, Georgia
Robinson, E., B.S., Harvard School of Public Health, Boston, Massachusetts
Schneider, A., Ph.D., University of California, Davis, MIND Institute, Sacramento
Scott, A., B.S., University of California, Los Angeles
Shepherd, S., B.S., Duke University, Durham, North Carolina
Van Peer, J., B.S., Leiden University, The Netherlands
Villalta-Gil, V., Ph.D., CIBERSAM-Saint John of God I+D Foundation, Sant Boi de Llobregat, Spain

SEMINARS

Adolphs, R., California Institute of Technology, La Jolla: The amygdala and social cognition.
Allman, J., California Institute of Technology, La Jolla: Neural specialization for primate social behavior.
Blakemore, S., University College London, United Kingdom: Workshop on the development of social cognition.
Bleakly, B., University of Exeter, United Kingdom: Guppies and what they tell us about social cognition.
Cahill, L., University of California, Irvine: Sex differences in emotion and social cognition.
Canli, T., Stony Brook University, New York: Imaging genomics.
Crawley, J., National Institutes of Heath, Bethesda, Maryland: Social deflects in mouse models of autism.
Fisher, S. University of Oxford, United Kingdom: Tangled webs: Tracing the connections between genes and cognition.
Fiske, S., Princeton University, New Jersey: Social neuroscience approaches to understanding other minds.
Goldberg, T., Zucker Hillside/Feinstein Institute/ECOM, Glenn Oaks, New York: Biology of mental illness: Deficits in social cognitive conference.
Insel, T., National Institutes of Mental Health/NIMH, Bethesda, Maryland: The future of research in social neuroscience.
Keysers, C., University of Groningen, The Netherlands: Mirror neurons and the human mirror system.
Marcus, G., New York University, New York: Language and the evolution of the human mind.
Moore, A., University of Exter, United Kingdom: Evolution of social behavior: What we can learn from insects.
Parr, L., Emory University, Yerkes National Primate Center, Atlanta, Georgia: Social cognition in chimpanzees.
Rangel, A., California Institute of Technology, La Jolla: An introduction to neuroeconomics.
Schultz, R., University of Pennsylvania, Philadelphia Children’s Hospital, Philadelphia: Face processing and the neural basis of autism.
Skuse, D., University College London, United Kingdom: X-linked genes and social cognition.
Tranel, D., University of Iowa, Des Moines: The frontal lobes and emotions.
Young, L., Emory University, Atlanta, Georgia: Pair bonding in voles.
Young, L., Harvard University, Cambridge, Massachusetts: Moral choices in the social brain.

Eukaryotic Gene Expression

July 16–August 5

INSTRUCTORS
J. Espinosa, University of Colorado, Boulder
W.L. Kraus, Cornell University, Ithaca, New York
T. Oelgeschlager, Marie Curie Research Institute, Oxted, United Kingdom
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri

ASSISTANTS
N. Gomes, University of Colorado, Boulder
B. Malecova, Marie Curie Research Institute, Oxted, United Kingdom
D. Ruhl, Cornell University, Ithaca, New York
M. Wu, Stowers Institute for Medical Research, Kansas City, Missouri

This course was designed for students, postdocs, and principal investigators who have recently ventured into the exciting area of gene regulation. The course focused on state-of-the-art strategies and techniques used in the field. Emphasis was placed on both in vitro and in vivo protein–DNA interactions and novel methodologies to study gene regulation. Students made nuclear extracts, performed in vitro transcription reactions, and measured RNA levels using primer extension. Characterizations of the DNA-binding properties of site-specific transcription factors were performed using electrophoretic mobility-shift and DNase I footprinting assays. In addition, students learned techniques for the assembly and analysis of chromatin in vitro.

During the past few years, the gene regulation field has developed in vivo approaches for studying gene regulation. In this course, students were exposed to the chromatin immunoprecipitation technique. They also used RNAi for specific knockdown experiments in mammalian cells. In addition, determining factor binding and gene expression profiles has accelerated tremendously by DNA microarray technology. Students received hands-on training in performing and interpreting results from ChIP-chip analyses and microarrays.

Experience with basic recombinant DNA techniques was a prerequisite for admission to this course. Lectures by the instructors covered the current status of the gene expression field, theoretical aspects of the methodology, and broader issues regarding strategies for investigating the regulation of gene expression in eukaryotes. Guest lecturers discussed contemporary problems in eukaryotic gene regulation and technical approaches to their solution.

Speakers in the 2008 course included Marisa Bartolomei (University of Pennsylvania School of Medicine), Wendy Bickmore (MRC Human Genetics Unit), Brian Dynlacht (New York University School of Medicine), Alexander Hoffmann (University of California, San Diego), Robert Kingston (Massachusetts General Hospital and Harvard Medical School), Tony Kouzarides (The Gurdon Institute), Michael Levine (University of California, Berkeley), Danesh Moazed (Harvard Medical School), Frank Pugh (Pennsylvania State University), Jeffrey Ranish (Institute for Systems Biology), Robert Roeder (The Rockefeller University), Michael Rosenfeld (University of California, San Diego), Patrick Schuiltz (IGBMC), Mike Snyder (Yale University), and Laszlo Tora (IGBMC).

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Alsheikhly Dantoft, W., M.S., Stockholm University, Sweden
Anckar, J., M.Sc., Turku Centre for Biotechnology, Finland
Bonthuis, P., B.S., University of Virginia, Charlottesville
Chatzi, K., B.S., Weill Cornell Medical College, New York
Chia, D., M.D., Oregon Health & Science University, Portland
Chuang, P., M.D., Mount Sinai School of Medicine, New York
de Araujo Souza, P., Ph.D., Brazilian National Cancer Institute, Rio de Janeiro
Elsaesser, S., Diploma, The Rockefeller University, New York
Franco, H., B.S., University of Puerto Rico School of Medicine, San Juan
Hekilmoglu, B., M.S., Institute of Molecular Biotechnology, Vienna, Austria
Kim, Y.J., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Lobanova, E., Ph.D., Duke University Medical Center, Durham, North Carolina
Munoz, M., M.S., LFBM IFIBYNE, CONICET, Buenos Aires, Argentina
Nuytemans, K., M.S., VIB: University of Antwerp, Belgium
Park, Y.P., Ph.D., National Institutes of Health, Rockville, Maryland
Pridalova, J., M.S., Institute of Molecular Genetics AS CR, Prague, Czech Republic

SEMINARS

Bartolomei, M., University of Pennsylvania School of Medicine, Philadelphia: Mechanism of genomic imprinting.
Bickmore, W., MRC Human Genetics Unit, Edinburgh, United Kingdom: Repressing gene expression at *Hox* loci, during differentiation and development.
Dynlacht, B., New York University School of Medicine and NYU Cancer Institute, New York: Transcriptional control of cell cycle exit and differentiation.
Hoffmann, A., University of California, La Jolla: Combinatorial and temporal codes of transcriptional activators.
Kingston, R., Massachusetts General Hospital and Harvard Medical School, Boston: New technologies to study the mechanisms of epigenetic regulation.
Kouzarides, T., University of Cambridge, United Kingdom: Chromatin modifying enzymes: Function and role in cancer.
Levine, M., University of California, Berkeley: Paved PO1II and development and cancer.
Moazed, D., Harvard Medical School, Boston, Massachusetts: RNAi and epigenetic inheritance of heterochromatin and cancer.
Pugh, F., Pennsylvania State University, University Park: Genomic organization of chromatin and the transcription machinery.
Ranish, J., Institute for Systems Biology, Seattle, Washington: Deciphering the composition of transcription complexes by quantitative mass spectrometry.
Roeder, R., The Rockefeller University, New York: Transcriptional activation mechanisms in animal cells: A largely biochemical approach.
Rosenfeld, M., University of California, San Diego, La Jolla: Regulatory networks in cellular growth and cancer development.
Schultz, P., IGBMC, Illkirch, France: Structural study of the eukaryotic transcription factor TFIID by electron microscopy and image analysis.
Snyder, M., Yale University, New Haven, Connecticut: Genomic analysis of gene expression and regulatory networks.
Tora, L., IGBMC, Illkirch, France: Toward the understanding of the regulation of RNA polymerase II transcription in a chromatin environment.

Imaging Structure and Function in the Nervous System

July 22–August 11

INSTRUCTORS	**S. Thompson,** University of Maryland School of Medicine, Baltimore **W. Tyler,** Arizona State University, Tempe **J. Waters,** Northwestern University, Chicago, Illinois
ASSISTANTS	**F. Albeanu,** MCB, Cambridge, Massachusetts **P. Baluch,** Arizona State University, Tempe **B. Clark,** Wolfson Institute for Biomedical Research, London, United Kingdom **M. Histed,** Harvard Medical School, Boston, Massachusetts **S. Hofer,** University College London, United Kingdom **A. Kampff,** Harvard University, Cambridge, Massachusetts **H. Mattison,** University of Maryland, Baltimore **S. Pal,** Harvard University, Cambridge, Massachusetts **B. Pichler,** University College London, United Kingdom **M. Rhiannon,** CNRC, Vrije Universiteit Amsterdam, The Netherlands **N. Takada,** Northwestern University, Chicago, Illinois **Y. Tufail,** Arizona State University, Tempe

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to use emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser-scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photoactivated (“caged”) compounds, and exocytosis tracers. Issues arising from the combination of imaging with electrophysiological methods were covered. Particular weight was given to multiphoton laser-scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. We used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells. Applicants had a strong background in the neurosciences or in cell biology.

Lecturers in the 2008 course included Hemant Bokil (Cold Spring Harbor Laboratory), Marcel

Bruchez (Carnegie-Mellon University), Karl Deisseroth (Stanford University), Winfried Denk (Max-Planck Institute for Medical Research), David DiGregorio (Universite Paris 5), Florian Engert (Harvard University), Oliver Griesbeck (Max-Planck Institute of Neurobiology), Samuel Hess (University of Maine), Mark Huebener (Max-Planck Institute of Neurobiology), David Kleinfeld (University of California, San Diego), Jurgen Klingauf (Max-Planck Institute for Biophysical Chemistry), Fred Lannie (Carnegie-Mellon University), Troy Margrie (University College London), Partha Mitra (Cold Spring Harbor Laboratory), Thomas Mrsic-Flogel (University College London), Venkatesh Murthy (Harvard University), Valentin Naegerl (Max-Planck Institute of Neurobiology), Timothy Ragan (Massachusetts Institute of Technology), Karel Svoboda (Howard Hughes Medical Institute), Philbert Tsai (University of California, San Diego), and Richard Weinberg (University of North Carolina).

This course was supported with funds provided by the National Institute of Mental Health (http://www.ninds.nih.gov/), the National Institute of Neurological Disorders & Stroke (http://www.ninds.nih.gov/), and the Howard Hughes Medical Institute (http://www.hhmi.org/).

Scientists from developing countries accepted into this course may be eligible for scholarships provided by the International Brain Research Organization. For details, go to http://www.iac-usnc.org/fellowships.htm.

PARTICIPANTS

Abrahamsson, T., M.S., CNRS, Paris, France
Alitto, H., Ph.D., University of California, Berkeley, Davis
Biro, A., M.D., University College London, United Kingdom
Branco, T., Ph.D., University College London, United Kingdom
Digby, G., M.S., Medical College of Georgia, Augusta
Frost, N., B.S., University of Maryland School of Medicine, Baltimore
Hong, Y.K., Sc.B., Harvard University, Cambridge, Massachusetts
Lloyd, T., Ph.D., Johns Hopkins School of Medicine, Baltimore, Maryland
Newton, J., Ph.D., University of California, San Francisco
Sun, D., Ph.D., Massachusetts General Hospital, Boston
Szatmari, E., Ph.D., Duke University, Durham, North Carolina
Viney, T., M.Biol., Friedrich Miescher Institute, Basel, Switzerland

SEMINARS

Bruchez, M., Carnegie-Mellon University, Pittsburgh, Pennsylvania: New and future indicators.
Deisseroth, K., Stanford University, California: Light-triggered activation/silencing.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: Extended two-photon imaging and block-face electron microscopy.
DiGregorio, D., Universite Paris 5, Paris, France and Thompson, S., University of Maryland School of Medicine, Baltimore, Maryland: Flash photolysis.
Engert, F., Harvard University, Cambridge, Massachusetts: Light sources (filaments, arc lamps, monchromators, metal halide lamps, LEDs, lasers, applications); Detectors (PMTs, CCDs, APDs, applications); Shot noise; Image J.
George, N., Olympus America, Inc., Center Valley, Pennsylvania: Objectives; BX51 optics.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Bayen, Germany: Genetically encoded sensors.
Hess, S., University of Maine, Orono: STORM, PALM, FPALM.
Huebener, M., Max-Planck Institute of Neurobiology, Martinsried, Germany: Intrinsic imaging.
Kilborn, K., Intelligent Imaging Innovations, Santa Monica, California: Deconvolution and frequency-domain FLIM.
Kleinfeld, D., University of California, San Diego, La Jolla: Spectral methods, denoising, and voltage-sensitive dyes.
Mitra, P., Cold Spring Harbor Laboratory: Numerical methods practical, Chronux.
Klingauf, J., Max-Planck Institute for Biophysical Chemistry, Gottingham, Germany: Fluorescence correlation microscopy.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Basic microscopy; DIC, Dodt tube, etc.
Litchman, J., Harvard University, Cambridge, Massachusetts: Confocal microscopy.
Margrie, T., University College of London, United Kingdom: Two-photon targeted patching.
Mrsic-Flogel, T., University College London, United Kingdom: Organic calcium indicators bulk loading.
Murthy, V., Harvard University, Cambridge, Massachusetts: Synaptofluorins and vesicle imaging.
Nagerl, V., Max-Planck Institute of Neurobiology, Munich, Germany: Nagerl's nuggets.
Prabhat, P., Semrock, Inc., Rochester, New York: Filters and dichroics.
Ragan, T., Massachusetts Institutes of Technology, Cambridge: Fluorescence block-face imaging.
Svoboda, K., Cold Spring Harbor Laboratory: Basics of two-photon imaging; Advanced two-photon:FRET, FLIM, two-photon uncaging.
Tsai, P., University of California, San Diego, La Jolla: Optics bench lab exercises: Basics Koehler; Optics bench lab exercises–Scanning; Optics bench lab exercises: Confocal.
Tyler, W., Arizona State University, Tempe: Imaging soundwaves.
Waters, J., Northwestern University, Chicago, Illinois: Homebrew microscope including laser safety; Organic calcium indicators; Single-cell calcium imaging in vivo.
Weinberg, R., University of North Carolina, Chapel Hill: Electron microscopy tomography.
Witkowski, J., Cold Spring Harbor Laboratory: On doing science: Ethics and responsible conduct.

Yeast Genetics and Genomics

July 22–August 11

INSTRUCTORS **B. Errede,** University of North Carolina, Chapel Hill
F. Luca, University of Pennsylvania, Philadelphia
M. Whiteway, National Research Council of Canada, Montreal

ASSISTANTS **P. Cote,** NRC Biotechnology Research Institute, Montreal, Canada
S. Fuchs, University of North Carolina, Chapel Hill
Y. Song, University of Pennsylvania, Philadelphia

This course is a modern, state-of-the-art laboratory course designed to teach 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 genetics 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 genetics interactions including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. Attendees gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, GFP–protein fusions, and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

This year's speakers included David Amberg (SUNY Upstate Medical University), David Botstein (Princeton University), Dan Burke (University of Virginia), Frederick Cross (The Rockefeller University),

Kara Dolinski (Princeton University), James Haber (Brandeis University), Mark Hochstrasser (Yale University), Anita Hopper (Ohio State University), Mark Johnston (Washington University Medical School), Mark Rose (Princeton University), Jeffrey Strathern (National Cancer Institute), Bik-Kwoon Tye (Cornell University), Lois Weisman (University of Michigan), and Reed Wickner (National Institutes of Health).

This course was supported with funds provided by National Human Genome Research Institute and the Howard Hughes Medical Institute (http://www.hhmi.org/).

PARTICIPANTS

Bao, Y., Ph.D., M.D. Anderson Cancer Center, Smithville, Texas
Bowman, B., Ph.D., Mills College, Oakland, California
Browne, C., B.S., Vanderbilt University School of Medicine, Nashville, Tennessee
Burwell, L., B.A., Cornell University, Ithaca, New York
Carter, G., Ph.D., Institute for Systems Biology, Seattle, Washington
Gruber, J., Ph.D., University of Michigan, Ann Arbor
Hughes, C., Ph.D., The Rockefeller University, New York
Johnson, C., B.S., Washington University, St. Louis, Missouri
Kilburn, C., B.A., University of Colorado, Boulder
Lai, K.C., B.S., Stowers Institute for Medical Research, Kansas City, Missouri
Luo, D., B.Sc., University of Birmingham, United Kingdom
McLaughlin, J., Ph.D., Rutgers University, New Brunswick, New Jersey
Mirkin, E., Ph.D., Harvard University, Cambridge, Massachusetts
Piotrowski, J., Ph.D., University of Montana, Missoula
Storey, J., Ph.D., Princeton University, New Jersey
Wang, G., Ph.D., University of North Carolina, Chapel Hill

SEMINARS

Amberg, D., SUNY Upstate Medical University, Syracuse, New York: Actin crazy in the Amberg lab.
Botstein, D., Princeton University, New Jersey: Coordination of growth rate, cell cycle, stress response, and metabolic activity in yeast.
Burke, D., University of Virginia, Charlottesville: Mitotic fidelity and infidelity…lessons from multiple checkpoints.
Cross, F., The Rockefeller University, New York: Yeast cell cycle.
Haber, J., Brandeis University, Waltham, Massachusetts: Checkpoint responses and repair of a broken chromosome.
Hochstrasser, M., Yale University, New Haven, Connecticut: Regulation of the yeast ubiquitin-proteasome system.
Hopper, A., Ohio State University, Columbus: Retrograde nuclear accumulation of cytoplasmic tRNA: A conserved response to nutrient deprivation.
Johnston, M., Washington University Medical School, St. Louis, Missouri: Feasting, fasting, and fementing: Glucose sensing and signaling in yeast.
Rose, M., Princeton University, New Jersey: Safe sex and the single cell.
Strathern, J., National Cancer Institute, Frederick, Maryland: The consequences of infidelity: Error-prone RNA polymerase.
Tye, B., Cornell University, Ithaca, New York: Modeling breast cancer in yeast.
Weisman, L., University of Michigan, Ann Arbor: Yeast vacuole inheritance: Insights into myosin-V-based movement and phosphoinositide signaling.
Wickner, R., National Institutes of Health, Bethesda, Maryland: Yeast prions: Biology and structure.

Mechanisms of Neural Differentiation and Brain Tumors

July 30–August 5

INSTRUCTORS
A. Guha, University of Toronto, Canada
E. Holland, Memorial Sloan-Kettering Cancer Center, New York
S. Majumuder, M.D. Anderson Cancer Center, Houston, Texas

ASSISTANT
S. Sinha, M.D. Anderson Cancer Center, Houston, Texas

This 1-week discussion course provided a clinical overview of brain tumors and emphasized molecular mechanisms involved in the growth and development of brain tumors with special emphasis on neural differentiation, signaling mechanisms, DNA replication, chromatin modulation, stem cells, mouse models, genomics, imaging techniques, genetically modified mouse techniques, nanotechnology, mechanism-based therapeutic strategies, and biobanks and ethical concerns. Attendees interacted with senior investigators on a one-to-one basis in an informal environment.

This course was supported with generous funding provided by the American Brain Tumor Association (http://www.abta.org/).

PARTICIPANTS

Agnihotri, S., B.S., Hospital for Sick Children Institute, Toronto, Canada
Antunes-Martins, A., Ph.D., University of Warwick, Coventry, United Kingdom
Bralten, L., B.S., Erasmus Medical Center, Erasmus University, Rotterdam, The Netherlands
Gu, P., Ph.D., M.D. Anderson Cancer Center, Houston, Texas
Hatton, B., B.S., Fred Hutchinson Cancer Research Center, Seattle, Washington
Huang, P., Ph.D., Massachusetts Institute of Technology, Cambridge

Jeyaretna, D., Ph.D., Massachusetts General Hospital, Boston
Kaarlander, M., B.S., Uppsala University, Sweden
Khabibullin, D., B.S., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Kim, B., Ph.D., University of Toronto, Ontario, Canada
Lafaille, F., B.S., Memorial Sloan-Kettering Cancer Center, New York
Maire, C., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Meley, D., Ph.D., University of Liverpool, United Kingdom
Schreck, K., B.S., Johns Hopkins University School of Medicine, Baltimore, Maryland
Smith, C., Ph.D., Hospital for Sick Children, Toronto, Canada
Song, H.-R., Ph.D., Children's Hospital of Los Angeles, University of Southern California, Los Angeles
Sussman, C., Ph.D., Mayo Clinic, Rochester, Minnesota
Wang, J., B.S., Beckman Research Institute of the City of Hope, Duarte, California
Wolf, A., B.S., Hospital for Sick Children, University of Toronto, Canada
Wu, X., Ph.D., Hospital for Sick Children, Toronto, Canada

SEMINARS

Burhans, W., Roswell Park Cancer Center, Buffalo, New York: DNA replication and cancer.
Cristea, I., Princeton University, New Jersey: Proteomic approaches to profiling a single CNS synapse type: A new role for phospholipid signaling in postsynaptic function.
Davis, F., University of Illinois, Chicago: Epidemiology of brain tumors.
Eberhart, C., National Cancer Institute, Baltimore, Maryland: Developmental signaling pathways, stem cells, and brain tumors.
Fuller, G., M.D. Anderson Cancer Center, Houston, Texas: Clinical overview: The role of neuropathology in contemporary diagnostic and experimental neuro-oncology.
Gilbertson, R., St. Jude's Children's Research Hospital, Memphis, Tennessee: How to cure brain tumors.
Gladson, C., University of Alabama, Birmingham: Regulation of migration and angiogenesis in gliomas.
Guha, A., Toronto Western Research Institute, Canada: Oncogenic mechanisms of EGFR mutants prevalent in GBMs.
Gutmann, D., Washington University School of Medicine, St. Louis, Missouri: Modeling brain tumors in mice: From cage to clinic.
Holland, E., Memorial Sloan-Kettering Cancer Center, New York: Trials and stem-like cells in mouse models of brain tumors.
Jain, R., Massachusetts General Hospital, Boston: Brain tumor angiogenesis and microenvironment.
James, C.D., University of California, Pacifica: Rodent enfragment/transplant models for the study of CNS tumors.
Kornblum, H., University of California Molecular and Medical Pharmacology, Los Angeles: Studying self-renewal in neural and brain tumor stem cells.
Lozano, G., University of Texas M.D. Anderson Cancer Center, Houston: The p53 tumor suppressor pathway.
Majumder, S., University of Texas M.D. Anderson Cancer Center, Houston: Stem cells in development/cancer.
Mietz, J., National Cancer Institute, Bethesda, Maryland: Funding opportunities at the National Institutes of Health.
Parada, L., University of Texas Southwest Medical Center, Dallas: Tumor suppressors (TSGs), oncogenes, and human cancers.
Pieper, R., University of California, San Francisco: Novel mechanisms of PTEN-mediated tumor suppression in gliomas.
Rich, J., Duke University Medical Center, Durham, North Carolina: Cancer stem cells and targeted therapies.
Rowitch, D., University of California, San Francisco: Hedgehog signaling in brain tumorigenesis.
Sawaya, R.H., University of Texas M.D. Anderson Cancer Center, Houston: When rocket science meets brain surgery.
Snyder, E., Burnham Institute, La Jolla, California: Some fundamental aspects of stem cell biology that might inform tumor pathophysiology.
Stiles, C., Dana-Farber Cancer Institute, Boston, Massachusetts: Transcription factors and malignant glioma in mouse and man.
Van den Bent, M., Daniel Den Hoed Cancer Center, Rotterdam, The Netherlands: Treatment of brain tumors: Depending on histology?
Wechsler-Reya, R., Duke University Medical Center, Durham, North Carolina: Uprooting hedges: The origin and propagation of medulloblastoma.
Weiss, W., University of California, San Francisco: PI3 kinase in tumors of the nervous system.
Yung, W.K.A., University of Texas M.D. Anderson Cancer Center, Houston: Molecular targeted therapies for glioblastoma.

Stem Cell Technologies

August 7–13

INSTRUCTORS	**E. Greiner,** Evotec AG, Hamburg, Germany **R. McKay,** National Institutes of Health, Bethesda, Maryland **M.M. Shen,** Columbia University Medical Center, New York
ASSISTANTS	**J. Chenoweth,** National Institutes of Health, Bethesda, Maryland

This 1-week lecture course brought together leading researchers in the stem cell field. 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, 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 was to provide participants with an opportunity to achieve an advanced understanding of the scientific and clinical importance of stem cells.

PARTICIPANTS

Abranches, E., Ph.D., Institute of Molecular Medicine, Lisbon, Portugal
Artus, J., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Burgold, T., B.S., Laboratory of Stem Cell Epigenetics, Milano, Italy
Fuchs, J., Ph.D., Children's Hospital of Pittsburgh, Pennsylvania
Gray, S., B.S., University of Dundee, United Kingdom
Jagadish, N., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Larsen, E., Ph.D., National Hospital, Oslo, Norway
Leeb, M., Ph.D., Institute of Molecular Pathology, Vienna, Austria
Ling, B., Ph.D., Stanford University, California
Narkis, G., Ph.D., National Institutes of Health, Bethesda, Maryland
Ougland, R., B.S., Massachusetts General Hospital/Harvard Medical School, Boston
Skotte, J., B.S., BRIC, University of Copenhagen, Denmark
Seidl, S., B.S., University of Oslo, Norway
Thomson, M., B.S., Harvard University/ Biophysics/System Biology, Cambridge, Massachusetts
Wessel Pederson, E., Ph.D., The Norwegian Radium Hospital, Oslo, Norway
Xie, W., B.S., University of California, Los Angeles

SEMINARS

Chaganti, R., Memorial Sloan-Kettering Cancer Center, New York: Stem cell and differentiation-specific transcription factor networks in germ cell tumors: Relevance to embryonic stem cells.
Chambers, I., University of Edinburgh, United Kingdom: ES cell self-renewal.
Dym, M., Georgetown University School of Medicine, Washington, D.C.: Male germ line stem cells I: Introduction and biology; Male germ line stem cells II: Future potential.
Farzad, M., Agilent Technologies, Washington, D.C.: Agilent Technologies demonstration.
Geijsen, N., Harvard Medical School/Massachusetts General Hospital, Boston: Different flavors of pluripotency.
Hadjantonakis, K., Memorial Sloan-Kettering Cancer Center, New York: Live imaging: Seeing is believing and revealing.
Heard, E., CNRS/ Institute Curie, Paris, France: X-chromosome inactivation: A model for epigenetic regulation during embryogenesis and in ES cells.
Hendrich, B., University of Cambridge, United Kingdom: Transcriptional control of stem cell fate.
Hochedlinger, K., Harvard Medical School, Boston, Massachusetts: Epigenetic reprogramming and induced pluripotency.
Impey, S., Oregon Health & Sciences University, Portland: Genomic approaches to the analysis of transcriptional and epigenetic networks.
McKay, R., National Institutes of Health, Bethesda, Maryland: Stem cells and Parkinson's disease.
Shen, M.M., Columbia University, New York: Nodal signaling and stem cells from the early mouse embryo.
Studer, L., Memorial Sloan-Kettering Cancer Center, New York: From epigenetic landscape to establishing lineage.
Surani, A., University of Cambridge, United Kingdom: The relationship between the germ line and pluripotent stem cells.
Testa, G., European Institute of Oncology, Milan, Italy: Epigenetic regulation by the Polycomb and Trithorax pathways: From genetic hints to molecular insight.
Yamaguchi, T., Center for Cancer Research, Frederick, Maryland: Wnt signaling, stem cells, and cell-fate determination in the mamamalian embryo.
Yu, H., Dana-Farber Cancer Institute, Boston, Massachusetts: Mapping interactions on a genomic scale.
Zernicka-Goetz, M., University of Cambridge, United Kingdom: Breaking symmetry in the mouse embryo: When, where, how.
Zwaka, T., Baylor College of Medicine, Houston, Texas: The role of Ronin in pluripotency and embryogenesis.

C. elegans

August 9–24

INSTRUCTORS	**S. Ahmed,** University of North Carolina, Chapel Hill **A. Desai,** University of California, San Diego, La Jolla **M. Zhen,** Samuel Lunenfeld Research Institute, Toronto, Canada
ASSISTANTS	**J. Calarco,** Samuel Lunenfeld Research Institute, Toronto, Canada **A. Dammermann,** University of California, San Diego, La Jolla **L. Shtessel,** University of North Carolina, Chapel Hill **L. Xie,** Samuel Lunenfeld Research Institute, Toronto, Canada

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 no experience with *C. elegans* and students with some prior worm experience who wished to expand their repertoire of expertise. The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction and screening of deletion libraries, and RNA inactivation. The course was designed to impart sufficient training to students in the most important attributes of the *C. elegans* system to enable them to embark on their own research projects after returning to their home institutions.

Speakers in the 2008 course included Alejandro Aballay (Duke University), Cori Bargmann (The Rockefeller University), Barbara Conradt (Dartmouth Medical School), Monica Driscoll (The State

University of New Jersey, Rutgers), David Fitch (New York University), Oliver Hobert (Columbia University College of Phycians & Surgeons), Erik Jorgensen (University of Utah), Donald Moerman (University of British Columbia), Amy Pasquinelli (University of California, San Diego), and Susan Strome (University of California, Santa Cruz).

This course was supported with funds provided by the National Institute of Child Health and Human Development (http://www.nichd.nih.gov/).

PARTICIPANTS

Ash, P., B.Sc., Mayo Clinic, Jacksonville, Florida
Biron, D., Ph.D., Brandeis/Harvard University, Cambridge, Massachusetts
Bois, J., Ph.D., MPI-CBG and MPI-PKS, Dresden, Germany
Bonini, N., University of Pennsylvania, Howard Hughes Medical Institute, Philadelphia
Eastwood, A., B.S., California Institute of Technology, Pasadena
Elle, I., B.Sc., University of Southern Denmark, Odense M, Denmark
Fang-Yen, C., Ph.D., Harvard University, Cambridge, Massachusetts
Giordano, R., M.Sc., Inserm, Bordeaux, France
Guillily, M., B.A., Boston University, Massachusetts
Kalb, R., M.D., Children's Hospital of Philadelphia, Pennsylvania
Kotak, S., Ph.D., University of Frankfurt, Germany
Leroy, M., M.S., Inserm, Paris, France
Leung, M., B.Sc., Duke University, Durham, North Carolina
Pollard, D., Ph.D., New York University, New York
Shimori, M., B.S., Kobe University, Japan
Wu, H.-Y., University of California, San Francisco

SEMINARS

Ahmed, S., University of North Carolina, Chapel Hill: Germ-line immortality.
Alejandro, A., Duke University, Durham, North Carolina: Pathogens and innate Immunity.
Arshad, D., University of California, San Diego, La Jolla: Cell division.
Bargmann, C., The Rockefeller University, New York: Olfaction and behavior.
Condradt, B., Dartmouth Medical School, Hanover, New Hampshire: Programmed cell death.
Driscoll, M., Rutgers University, Piscataway, New Jersey: Mechanosensation, neuronal death, and aging.
Fitch, D., New York University, New York: Evolution of *Caenorhabditis* and other rhabditid nematodes.
Hobert, O., Columbia University, New York: microRNA and neuronal left-right asymmetry.
Jorgensen, E., University of Utah, Salt Lake City: A simple behavioral circuit.
Moerman, D., University of British Columbia, Vancouver, Canada: Functional genomics.
Pasquinelli, A., University of California, San Diego, La Jolla: microRNA biogenesis and function.
Strome, S., University of California, Santa Cruz: Germ-line gene expression.
Zhen, M., Samuel Lunenfeld Research Institute, Toronto, Canada: Development of the nervous system.

X-ray Methods in Structural Biology

October 13–28

INSTRUCTORS	**W. Furey,** V.A. Medical Center and University of Pittsburgh, Pennsylvania **G. Gilliland,** Centorcor R&D, Inc., Radnor, PA **A. McPherson,** University of California, Irvine **J. Pflugrath,** Rigaku Americas Corporation, The Woodlands, Texas
ASSISTANT	**M. Thomas,** Centocor R&D, Inc., Radnor, Pennsylvania

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensified laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics covered included basic diffraction theory, crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction, molecular replacement, solvent flattening, noncrystallographic symmetry averaging, electron density interpretation, molecular graphics, structure refinement, structure validation, coordinate deposition, and structure presentation. Participants learned through extensive hands-on experiments. One or more proteins were crystallized and the structure(s) determined by several methods, in parallel with lectures on theory and informal discussions behind the techniques. Applicants were familiar with the creation and editing of simple text files on Linux workstations using a screen-based editor (either vi or emacs).

Speakers in the 2008 course included Paul Adams (Lawrence Berkeley Laboratory), Serge Cohen (Netherlands Cancer Institute, Amsterdam), Zbigniew Dauter (Agronne National Laboratory), Paul Emsley (University of York, United Kingdom), Hiro Furukawa (Cold Spring Harbor Laboratory), Jeffrey

Headd (Duke University Medical Center), Wayne Hendrickson (Columbia University), Xinhua Ji (National Cancer Institute), Leemor Joshua-Tor (Cold Spring Harbor Laboratory), Gerard Kleywegt (University of Uppsala, Sweden), Anastassis Perrakis (Netherlands Cancer Institute), Randy Read (University of Cambridge, United Kingdom), David Richardson (Duke University Medical Center), Robert Sweet (Brookhaven National Laboratory), Thomas Terwilliger (Los Alamos National Laboratory), Dale Tronrud (University of Oregon), John Westbrook (State University of New Jersey, Rutgers), and Michael Wiener (University of Virginia).

This course was supported with funds provided by the National Cancer Institute (http://www.cancer.gov/) and the Howard Hughes Medical Institute (http://www.hhmi.org/).

PARTICIPANTS

Allerston, C., Ph.D., University College London, United Kingdom
Ash, M.-R., B.S., University of Sydney, Australia
Economou, N., Diploma, Drexel University, Philadelphia, Pennsylvania
Fanucchi, S., Ph.D., University of the Witwatersrand, Gauteng, South Africa
Gerratana, B., Ph.D., University of Maryland, College Park
Gilbert, N., B.S., Louisiana State University, Baton Rouge
Guttler, T., M.S., Max-Planck Institute, Gottingen, Germany
Hibbs, R., Ph.D., Oregon Health & Science University, Portland
Hook, P., Ph.D., Columbia University, New York
Hrmova, M., Ph.D., University of Adelaide, Glen Osmond, Australia
Knauer, S., University of Bayreuth, Germany
Krey, T., Ph.D., Institut Pasteur, Paris, France
Luo, X., Ph.D., University of Texas Southwestern Medical Center, Dallas
Napetschnig, J., M.Sc., The Rockefeller University, New York
Robinson, P., Stanford University, California
Tidow, H., Ph.D., MRC Centre for Protein Engineering, Cambridge, United Kingdom
Viani Puglisi, E., Ph.D., Stanford University School of Medicine, California

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, Berkeley, California: Structure refinement.
Cohen, S., Netherlands Cancer Institute, Amsterdam, The Netherlands: Automated structure solution and model building.
Dauter, Z., Agronne National Laboratory, Illinois: Anomalous data collection.
Emsley, P., University of York, United Kingdom: Model-building tools in cool.
Furukawa, H., Cold Spring Harbor Laboratory: Structure and function of NMDA receptors.
Hendrickson, W., Columbia University, New York: MAD phasing: Theory and practice.
Ji, X., National Cancer Institute, Frederick, Maryland: Structure of RapA, a Swi2/Snf2 protein that recycles RNA polymerase during transcription.
Joshua-Tor, L., Cold Spring Harbor Laboratory: DNA translocation: One step at a time; Structure presentation.
Kleywegt, G., University of Uppsala, Sweden: Just because it's in Nature, doesn't mean it's true…(macromolecular structure validation).
Read, R., University of Cambridge, United Kingdom: Molecular replacement: New structures from old; Using SAD data in phaser.
Richardson, D., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using MolProbity.
Sweet, R., Brookhaven National Laboratory, Upton, New York: Fundamental of crystallography; X-ray sources and optics.
Terwilliger, T., Los Alamos National Laboratory, New Mexico: Automated structure solution and model building.
Tronrud, D., University of Oregon, Eugene: Difference electron density maps; Macromolecular refinement I; Macromolecular refinement II.
Westbrook, J., The State University of New Jersey, Rutgers, Pisacataway: Automating PDB deposition.
Wiener, M., University of Virginia, Charlottesville: Membrane protein crystallization.

Programming for Biology

October 15–28

INSTRUCTORS
S. Lewis, University of California, Berkeley
S. Prochnik, DOE–Joint Genome Institute/University of California, Berkeley
J. Tisdall, DuPont Experimental Station, Wilmington, Delaware

ASSISTANTS
D. Curiel, Pioneer Hi-Bred, Johnston, Iowa
E. Lee, Lawrence Berkeley National Laboratory, Berkeley, California
J. Leipzig, DuPont Experimental Station, Wilmington, Delaware
J. Karalius, Seminis Vegetable Seeds, Inc., Woodland, California
D. Messina, Stockholm University, Sweden
K. Norwick, University of Illinois, Urbana-Champagne
S. Robb, University of Utah, Salt Lake City

A computer is already an indispensable tool for database searches, but the use of web-based tools alone is not enough for today's biologist who needs to access and work with data from myriad sources in disparate formats. This need has become ever more important as new technologies have increased the already exponential rate at which biological data is generated. Designed for students and researchers with little or no prior programming experience, this 2-week course gave biologists the bioinformatics skills necessary to exploit the abundance of biological data.

The course was based around the Perl scripting language, because of its ease of learning and its incredible wealth of ready-built code modules (e.g., bioperl) designed to solve common biological problems. Starting with introductory coding, and continuing with a survey of available biological libraries and practical topics in bioinformatics, students ended by learning how to construct and run powerful and extensible analysis pipelines in a straightforward manner. The course combined formal lectures with hands-on sessions in which students worked to solve problem sets covering common scenarios in the acquisition, validation, integration, analysis, and visualization of biological data. For their final projects,

which ran during the second week of the course, students were posed with problems using their own data and worked with one another and the faculty to solve them. Final projects formed the basis of publications as well as public biological websites (see, e.g., http://bio.perl.org/wiki/Deobfuscator).

The prerequisites for the course were basic knowledge of UNIX. Lectures and problem sets covering this background material were available online from previous years and students studied this material before starting the course. The primary focus of this course was to provide students with practical programming experience, rather than to present a detailed description of the algorithms used in computational biology. For the latter, we recommend the Computational Genomics course (http://meetings.cshl.edu/courses/c-ecg08.shtml).

Speakers in the 2008 course included Scott Cain, Roderic Guigo, Winston Hide, Curtis Huttenhower, Sheldon McKay, William Pearson, Jason Stajich, Lincoln Stein, and Paul Thomas.

This course was supported by the National Human Genome Research Institute (http://www.genome.gov/).

PARTICIPANTS

Blouin, N., M.S., University of Maine, Orono
Boucher, Y., Ph.D., Massachusetts Institute of Technology, Cambridge
Boysen, J., B.S., New York University School of Medicine/Skirball Institute, New York
Ellison, C., B.A., University of California, Berkeley
Eveland, A., Ph.D., Cold Spring Harbor Laboratory
Fagerberg, L., M.Sc., Royal Institute of Technology, Stockholm, Sweden
Faria, R., Ph.D., Universitat Pompeu, Fabra, Barcelona, Spain
Fievet, B., Ph.D., Gurdon Institute/University of Cambridge, United Kingdom
Gongora-Castillo, E., Cinvestav, Irapuato, Mexico
Gruntman, E., M.S., Cold Spring Harbor Laboratory
Irvine, D., B.S., Cold Spring Harbor Laboratory
Jimenez-Gomez, J., Ph.D., University of California, Davis
Krause, J., Diploma, Max-Planck Institute, Leipzig, Germany
Li, W., B.S., Cold Spring Harbor Laboratory
Metsugi, S., M.S., The Institute for Advanced Study, Princeton, New Jersey
Meyer, M., Diploma, Max-Planck Institute, Leipzig, Germany
Ortiz-Pineda, P., B.S., University of Puerto Rico, San Juan
Perry, M., Ph.D., Ontario Institute for Cancer Research, Canada
Ramsey, J., B.A., Cornell University, Ithaca, New York
Rodriquez, J., Ph.D., The Gurdon Institute, Cambridge, United Kingdom
Rymarquis, L., Ph.D., University of Delaware, Newark
Woods, I., B.S., Harvard University, Cambridge, Massachusetts
Zhuang, Y., B.S., University of Connecticut, Groton

SEMINARS

Cain, S., Cold Spring Harbor Laboratory: gbrowse: The generic genome browser.
Guigo, R., University Pompeu Fabra, Spain: Gene prediction.
Hide, W., Harvard University, Massachusetts: EST sequence clustering and analysis.
Huttenhower, C., Princeton University, New Jersey: Microarrays.
McKay, S., Cold Spring Harbor Laboratory: DB design + SQL; DBI; HTML; CGI.
Pearson, W., University of Virginia, Charlottesville: Sequence similarity.
Stajich, J., University of California, Berkeley: Bioperl I; Bioperl II.
Thomas, P., SRI International, Menlo Park, California: Protein function prediction.

Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 19–November 1

INSTRUCTORS
V. Allan, University of Manchester, United Kingdom
K. Hu, Indiana University, Bloomington
S. Huang, Northwestern University School of Medicine, Chicago, Illinois
J. Murray, University of Pennsylvania School of Medicine, Philadelphia

ASSISTANTS
P. March, University of Manchester, United Kingdom
C. Wang, University of California, Davis
M. Wozniak, University of Manchester, United Kingdom

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. It was intended 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, 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.

With 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.

Speakers in the 2008 course included Michael Davidson, Richard Day, Joseph Gall, Alexey Khodjakov, Alison North, and Thomas Ried.

This course was supported with funds provided by the National Cancer Institute (http://www.cancer.gov/).

PARTICIPANTS

Bair, A., Ph.D., Harvard University/Massachusetts General Hospital, Charlestown
da Fonseca Pereira, C., Ph.D., Macfarlane Burnet Institute, Melbourne, Australia
Deglincerti, A., Laureate, Weill Cornell Medical College, New York
George, P., B.S., Virginia Tech, Blacksburg
Gilman, J., B.S., University of Colorado, Denver
Hsiao, C.-J., Ph.D., University of Connecticut, Storrs
Jurcisek, J., B.S., The Research Institute at Nationwide Children's Hospital, Columbus, Ohio
Miller, J., Ph.D., University of Iowa, Iowa City
Mullers, E., B.A., Dresden International Graduate School, Germany
Parkhitko, A., M.S., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Plotnikova, O., M.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Recino, A., M.S., University of Bath, United Kingdom
Schmidt, C., Dipl., Cancer Research UK, London, United Kingdom
Vijayakumar, S., Ph.D., New York University Medical Center, New York
Wong, G., B.S., Cambridge University, United Kingdom
Zhu, Y., Ph.D., The University of Melbourne, Australia

SEMINARS

Allan, V., University of Manchester, United Kingdom: Immunocytochemistry.
Davidson, M., The Florida State University, Tallahassee: A library of fluorescent proteins for live cell imaging.
Day, R., University of Virginia, Charlottesville: Seeing colors: Applications and limitations of the fluorescent proteins.
Gall, J., Carnegie Institution, Baltimore, Maryland: The development of in situ hybridization.
Hu, K., Indiana University, Bloomington and Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Introduction to light and fluorescence microscopy.
Huang, S., Northwestern University School of Medicine, Chicago, Illinois: Introduction to FISH.
Khodjakov, A., Wadworth Center, E. Greenbush, New York: Control of daughter centriole formation.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Digital detectors and digital imaging fundamentals; Microscopy of thick specimens.
North, A., The Rockefeller University, New York: Immunocytochemistry on tissue sections principles and practices.
Ried, T., National Cancer Institute/National Institutes of Health, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Spector, D., Cold Spring Harbor Laboratory: Visualizing gene expression/silencing in living cells.

Computational and Comparative Genomics

November 5–11

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
R. Smith, GlaxoSmithKline, King of Prussia, Pennsylvania

ASSISTANT **D. Triant,** Louisiana State University, Baton Rouge

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. It 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 world wide web pages to present problem sets and the computing tools to solve them. Students used Windows and Mac workstations attached to a UNIX server; participants were 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 advanced software development were encouraged to apply to the course on Programming for Biology (http://meetings.cshl.edu/courses/c-info07.shtml).

Speakers in the 2008 course included Stephen Altschul (National Library of Medicine), Michael Ashburner (European Bioinformatics Institute), Peter Cooper (NCBI/NLM), Bert Overduin (European Bioinformatics Institute), and Lisa Stubbs (University of Illinois).

This course was supported by the National Human Genome Research Institute (http://www.genome.gov/).

PARTICIPANTS

Arighi, C., B.S., Protein Information Resource, Washington, D.C.
Ayub, M., B.S., University of Dhaka, Bangladesh
Burbano, H., M.Sc., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Cao, J., Ph.D., Wyeth Research, Cambridge, Massachusetts
DeBarry, J., B.S., University of Georgia, Athens
Fatakia, S., B.S., NIDDK/National Institutes of Health, Bethesda, Maryland
Glass, S., M.S., Wyeth Research, Cambridge, Massachusetts
Grosso, A.R., B.S., Lisbon University, Portugal
Moorthy, S., Ph.D., Perkasie, Pennsylvania
Pich, C., Ph.D., ZFIN, University of Oregon, Eugene
Roltgen, K., B.S., University of Basel, Switzerland
Ross, E., M.B.A., University of Utah, Salt Lake City
Silva, L., B.S., Rene Rachou Research Center, Belo Horizonte, Brazil
Spinner, W., B.Sc., University of Bristol, United Kingdom
Szatkiewicz, J., Ph.D., The Jackson Laboratory, Bar Harbor, Maine
Tang, Y.L., Ph.D., Keck Graduate Institute of Applied Life Science, Claremont, California

SEMINARS

Altschul, S., National Library of Medicine, Bethesda, Maryland: Statistics of sequence similarity scores; Iterated protein database searches with PSI-BLAST.
Ashburner, M., European Bioinformatics Institute, Hinton, Cambridge, United Kingdom: Biological ontologies.
Cooper, P., NCBI/NLM, Bethesda, Maryland: NCBI resources for bioinformatics and computational biology; NCBI genome resources.
Nowick, K., University of Illinois, Urbana: Analysis of zinc finger expansion in vertebrate genomes.
Overduin, B., European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom: The ENSEMBL database of genomes I; ENSEMBL II.
Pearson, W., University of Virginia, Charlottesville: Alignment algorithms: Large-scale alignment; Protein evolution and sequence similarity searching; Algorithms for biological sequence comparison; Hidden Markov models and protein profiles; Identifying consensus sites.
Smith, R., GlaxoSmithKline, King of Prussia, Pennsylvania: Approaches to multiple sequence alignment/multiple alignment resources.
Stubbs, L., University of Illinois, Urbana: Genome comparison biology.

Phage Display of Proteins and Peptides

November 6–19

INSTRUCTORS
C. Barbas, The Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, University of California, San Diego, La Jolla

ASSISTANTS
S. Kacir, University of Pennsylvania, Philadelphia
I. Molano, Medical University of South Carolina, Charleston
K. Noren, New England BioLabs, Ispwich, Massachusetts

Recent advances in the generation and selection of antibodies from combinatorial libraries allowed 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 *Escherichia coli* were also covered. Epitopes were selected from peptide libraries and characterized.

The lecture series, presented by a number of invited speakers, emphasized PCR of immunoglobulin genes, the biology of filamentous phage, the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, directed protein evolution, retroviral and cell display libraries, the immunobiology of the antibody response, and recent results on the use of antibodies in therapy. The theory and practical implications for selection from phage-displayed libraries of random peptides, cDNA products, and semisynthetic proteins were also explored.

Seminar speakers in the 2008 course included Angela Belcher (Massachusetts Institute of Technology), Germaine Fuh (Genentech, Inc.), Christopher Noren (New England BioLabs), Christoph Rader (National Institutes of Health), Nathalie Scholler (University of Pennsylvania), Sachdev Sidhu (University of Toronto), George Smith (University of Missouri), and Robyn Stanfield (The Scripps Research Institute).

This course is supported with funds provided by the Howard Hughes Medical Institute (http://www.hhmi.org/).

PARTICIPANTS

Amara, I., M.S., Institute of Biotechnology, UNAM, Cuernavaca, Mexico
Axelsson, F., M.Sc., Lund University, Sweden
Capparelli, F., M.S., Federial University of Uberlandia, Brazil
Chang, K.-H., M.S., Green Cross Corporation, Yongin, South Korea
Conroy, P., Ph.D., Dublin City University, United Kingdom
Ferrer-Miralles, N., Ph.D., Autonomous University of Barcelona, Spain
Gomes, K.N., B.S., University of Sao Paulo, Brazil
Kimura, M., Ph.D., Gifu University School of Medicine, Japan
Kivil, A., M.A., Tallinn University of Technology, Estonia
Lam, A., B.A., OncoMed Pharmaceutical, Inc., Redwood City, California
Morales, C., B.S., Trubion Pharmaceuticals, Seattle, Washington
Neelamegar, S., M.Sc., Invitrogen, Bangalore, India
Nilvebrant, J., M.Sc., Royal Institute of Techology, Stockholm, Sweden
Wadas, T., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Woelfle, M., M.D., The Feinstein Institute for Medical Research, Manhasset, New York
Yang, M., Ph.D., Canadian Food Inspection Agency, Winnipeg, Canada

SEMINARS

Barbas, C., The Scripps Research Institute, La Jolla, California: Developing therapeutic strategies with phage display.
Belcher, A., Massachusetts Institute of Technology, Cambridge: Phage as a toolkit for the synthesis and assembly of materials for electronics and energy.
Fuh, G., Genentech, Inc., S. San Francisco, California: Synthetic human antibodies and their use in biology.
Noren, C., New England BioLabs, Beverly, Massachusetts: Phage peptide libraries: The Ph.D. for peptides.
Rader, C., National Cancer Institute/National Institutes of Health, Bethesda, Maryland: Mining human antibody repertoires by phage display.
Scholler, N., University of Pennsylvania, Philadelphia: Yeast display.
Sidhu, S., University of Toronto, Canada: 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, La Jolla: Repertoire cloning of SLE autoantibodies.
Smith, G., University of Missouri, Columbia: Principles of affinity selection; Massively parallel selections.
Stanfield, R., The Scripps Research Institute, La Jolla, California: Structural biology of the immune system.

The Genome Access Course

April 29–30, November 18–19

TRAINERS **G. Howell,** The Jackson Laboratory, Bar Harbor, Maine
B. King, The Jackson Laboratory, Bar Harbor, Maine
L. Reinholdt, The Jackson Laboratory, Bar Harbor, Maine
R. Sachidanandam, Mount Sinai School of Medicine, New York

This course is an intensive 2-day introduction to bioinformatics that was held twice in 2008 and trained almost 43 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: 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 Genome Research Center at Woodbury, which is 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.

April 29–30

Allen, M., Pfizer Inc., Groton, Connecticut
Barral, J., The University of Texas Medical Branch, Galveston
Du, R., Harvard Medical School/Brigham and Women's Hospital, Boston, Massachusetts
Elias, L., University of California, San Francisco
Etzrodt, M., Massachusetts General Hospital, Charlestown
Gladdy, R., Memorial Sloan-Kettering Cancer Center, New York
Hebert, T., McGill University, Montreal, Canada
Hendrickson, M., Syosset Central School District, New York
Kaiser, C., The University of Texas Medical Branch, Galveston
Kim, H., Roswell Park Cancer Institute, Buffalo, New York
Kurland, I., State University of New York, Stony Brook
Laing, R., University of Glasgow, United Kingdom
Laurindo, F., Heart Institute, University São Paulo School Medicine, Brazil
Naidu, D., State University of New York, Stony Brook
Oswald, M., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Padhi, B., Health Canada, Ottawa
Sezen, U., University of Connecticut Ecology and Evolutionary Biology, Storrs
Shoelson, B., The MathWorks, Natick, Massachusetts

November 18–19

Cardone, L., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Crummett, C., Massachusetts Institute of Technology, Cambridge
Debette, S., Boston University, Framingham Heart Study, Boston, Massachusetts
Erblich, J., Mount Sinai School of Medicine, New York
Fox, A., Brigham and Women's Hospital Harvard Medical School, Boston, Massachusetts
Ghiban, C., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Gottwein, E., Duke University Medical Center, Durham, North Carolina
Guffanti, G., University of California, Irvine
Jimenez, R., Brown University, Providence, Rhode Island
June, R., University of California, San Diego
Lim, S., Bonn Medical School, Bonn, Germany
Moon, B., Columbia University, New York
O'Grady, T., Mount Sinai School of Medicine, New York
Parr, R., Texas A&M University, College Station
Pietrzykowski, A., University of Massachusetts, Worcester
Platero, H., Stanford University, Palo Alto, California
Plavskin, Y., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Rebolini, D., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Salih, S., University of Wisconsin, Madison
Sotos, M., Valencia University, Valencia, Spain
Svoren, E., William Harvey Research Institute, London, United Kingdom
Tang, K.-W., University of Göteburg, Sweden
Trimble, J., St. Francis University, Loretto, Pennsylvania
Yun, C., New York University School of Medicine, New York
Zhang, Z., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

SEMINARS

INVITED SPEAKER PROGRAM

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their findings. These weekly seminars keep the CSHL staff current on the latest scientific developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

	Title	Host
January		
Dr. William Kaelin, Harvard University/Dana Farber Cancer Institute	The VHL tumor suppressor gene: Oxygen sensing and cancer	Scott Lowe
Dr. David Kingsley, Stanford University School of Medicine	Fishing for the secrets of vertebrate evolution	Scott Powers
February		
Dr. Leslie Vosshall, The Rockefeller University	How insects sense odor concentration with ion channels	Scott Powers
Dr. Jonathan Widom, Northwestern University	The genomic code for nucleosome positioning	Leemor Joshua-Tor
March		
Dr. Eileen White, Rutgers University	Role of autophagy in cancer	Terri Grodzicker
Dr. Benjamin Cravatt, The Scripps Research Institute	Mapping dysregulated biochemical pathways in cancer by integrated proteomics and metabolomics	Leemor Joshua-Tor
Dr. Marc Vidal, Dana-Farber Cancer Institute/Harvard Medical School	Interactome networks and human disease	David Jackson
Dr. Richard Caprioli, Vanderbilt University	Molecular imaging and profiling of tissue sections using mass spectrometry: Applications in biological and clinical research	Leemor Joshua-Tor
April		
Dr. David Anderson, California Institute of Technology	Neural circuits for innate defensive behaviors in flies and mice	Zach Mainen
Dr. Robert Kingston, Harvard Medical School/Massachusetts General Hospital	Unraveling the genome using new technologies	Terri Grodzicker
October		
Dr. Chris Q. Doe, University of Oregon	Cell polarity and spindle orientation regulate *Drosophila* stem cell self-renewal	Senthil Muthuswamy
Dr. Charles Zuker, University of California, San Diego	Common sense about taste—From the tongue to the brain	Bruce Stillman
November		
Dr. Tobias Meyer, Stanford University	Logics of signaling systems that control Ca^{2+} homeostasis and cell migration	Lloyd Trotman
Dr. Victor Ambros, University of Massachusetts Medical School	microRNA pathways in animal development	Gregory Hannon
December		
Dr. Rob Phillips, California Institute of Technology	A single-molecule view of biological action at a distance	Leemor Joshua-Tor
Dr. M. Geoffrey Rosenfeld, University of California, San Diego	Life, death, and transformation: Movement, DNA repeats, and ncRNAs	Gregory Hannon

IN-HOUSE SEMINAR PROGRAM

Cold Spring Harbor Laboratory's In-House Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

	Title
January	
Jan Witkowski	Eugenics, immigration, and the politicizing of science
Martin Bayer (Lukowitz Lab)	Paternal control of embryonic patterning in *Arabidopsis thaliana*
Bill Keyes (Mills Lab)	p63 regulates stem cells in cancer and aging
February	
Gidon Felsen (Mainen Lab)	Neural circuits for decision-making in rats
Michael Huebner (Spector Lab)	Silence, please! Polycomb proteins and gene regulation
Josh Huang	Construction and function of the GABA inhibitory circuits
Jonathan Sebat	Analysis of genome copy-number variation in schizophrenia
Jen Bestman (Cline Lab)	The RNA-binding protein CPEB controls dendrite growth and neural circuit assembly in vivo
March	
Milos Tanduric (Martienssen Lab)	Selective silence: Epigenomic variation and fluctuation in plants
Anne Daulny (Tansey Lab)	Negative regulation of RNA polymerase II by ubiquitylation
Josh Dubnau	Memories of a fly: Dissection with vegetables and dogs
April	
Zi Zhong	Understanding of memory through Benzer's eyes
Doreen Ware	Interpreting plant genomes, a comparative journey
Lincoln Stein	Genome annotation: Farmer's mart or Wal-Mart?"
October	
Valery Krizhanovsky (Lowe Lab)	Cellular senescence beyond tumor suppression
Partha Mitra	Evolution of song culture in the Zebra Finch
Yoselin Benitez Alfonso (Jackson Lab)	Reactive oxygen species regulate intercellular communication during plant development
Jeremy Wilusz (Spector Lab)	Looking beyond classical polyadenylation: How a noncoding RNA uses the tRNA processing machinery to defy the dogma
November	
Xavier Roca (Krainer Lab)	New mechanisms of splice-site recognition: Implications for genomics and disease
December	
Emily Hodges (Hannon Lab)	CliffsNotes to the genome—A portable guide for probing cancer, the epigenome, and ancient DNA
Michael Zhang	Large-scale network-based prediction of human disease genes
Avi Rosenberg (Muthuswamy Lab)	Cell shape meets cell fate in the breast

BANBURY CENTER

BANBURY CENTER EXECUTIVE DIRECTOR'S REPORT

The year 2008 was unusually quiet at Banbury. Our meetings season was much shorter than usual, finishing at the end of October. It was then that we began a major renovation of Robertson House that necessitated emptying the house and turning it over to the Laboratory's facilities crews and outside contractors. Times have changed and conditions that were once acceptable are no longer so. Most significantly, air-conditioning is being installed—an upgrade that will be much appreciated by all those who will spend the summer months here! We are taking advantage of this disruption to rewire the house, redecorate, and upgrade Internet facilities. The house was built in 1936 so we will be in good time to celebrate its 75th anniversary in 2011.

As a consequence of the shortened season, there were only 18 scientific meetings in 2008 and a correspondingly smaller number of participants: 524. Of these, 81% came from the United States, specifically from 33 states, with New York, California, Massachusetts, and Maryland providing most of these participants. Almost one-fifth of participants came from outside of the United States, from 16 countries. In addition to the meetings, there were six CSHL lecture courses, and two CSHL laboratories held retreats here. We were happy to again welcome local organizations including the Conservation Board of the Village of Lloyd Harbor, the Cold Spring Harbor School District, and the Caumsett Foundation. So, overall, the Center was busy throughout the year.

The year 2008 marked Jim Watson's 40th year at Cold Spring Harbor Laboratory, and to observe the occasion, Banbury Center held three meetings on topics of special interest to Jim. The common thread of the meetings involved topics of interesting scientific research, the results of which have important consequences for society and should help form public policy. The titles of each were cast as questions, in the expectation that this phrasing would help focus minds and provoke debate. Regrettably, but not surprisingly, we did not answer any of the questions. However, the meetings brought together fascinating groups of people for very lively discussions.

Coffee break discussions

The first of the three meetings was entitled *How Will We Prevent Most Forms of Cancer?* We have been performing research on cancer for more than a century and since the early 1970s, we have discovered many of the genetic and biochemical changes that turn a normal cell into a cancer cell. This knowledge is going to increase rapidly with the application of large-scale genomic analysis of tumors. However, despite this intensive research, the number of cancers that can at present be cured effectively and efficiently remains small. The participants explored why this is so, evaluated current and potential therapies, asked what can be done to improve early detection, and faced the issue that some cancers may not be curable.

The second meeting in the series asked the question *To What Age Should We Be Expected to Work?* Here, the scope of the discussions ranged from laboratory research on aging through to the economic consequences of extended life span. Considerable progress has been made in elucidating some of the genetic changes and molecular processes that contribute to aging in experimental organisms. Although our lives are being extended through better lifestyles and improved health care, it seems, however, that our maximum life spans are unchanged. Is extending the human life span an achievable or desirable goal? Should we concentrate instead on maintaining the quality of life for the extra years that we are gaining now? That is, can we live longer and still be healthy?

The third meeting was also of broad scope, ranging from research to social impact to educational policy: *How Can We Improve Our Brains?* It is the hope of every parent that their child will be bright and intelligent. Parents work to help their children's brain work better by providing education and stimulation, and society as a whole makes a tremendous commitment to the education of its young people. Are there data emerging from cognitive neuroscience that such education programs should take into account? Are there learning regimes that might be more effective than those typically found in the classroom? At the other end of life is the hope of all of us that the normal decline in cognitive skills that accompanies aging will be slow. Might "brain exercises" maintain our brains at a higher level of functioning? Is there evidence that such exercises work? Are there effective pharmacological agents? In short, how can we best use the resources of society to help our brains work better throughout our lives?

Meetings dealing with biomedical research that has important social implications seem to have been the norm at Banbury in 2008. *Who Are We? Kinship, Ancestry, and Social Identity* reviewed a field that has been changing rapidly because of technical advances in genomics and rapidly increasing public interest. Participants discussed the issue that although genetics provides a description of human beings that reflects their *biological* ancestry, cultural norms provide a description of *social* ancestry, and these two descriptions need not be, and often are not, the same. This has been highlighted by the increasing use of DNA-based analysis for tracing genealogical ancestry. Here, discrepancies between ancestry revealed by genetic analysis and assumed by cultural descent may profoundly affect individuals' views of themselves. The importance of the relationship between genetic and ethnic identities requires careful, rational, and critical review of what is a controversial subject.

I began my research career working on Duchenne's muscular dystrophy (DMD), studying cells growing in tissue culture. At that time, no human genes, let alone genes involved in genetic disorders, had been isolated, and the cloning in the mid 1980s of the gene involved in DMD was a landmark in human genetics. It was confidently expected, now that the protein was known and the gene was available for gene therapy, that treatments would quickly follow, but that optimism was misplaced. In the succeeding years, we have learned that going from a cloned gene to a therapy is extraordinarily difficult. However, a new therapeutic strategy has been developed that uses oligonucleotides to direct the splicing of an mRNA so that a functional or partially functional protein is produced. This has proved to be successful in experimental models of DMD and spinal muscular atrophy. Participants in the meeting *Oligonucleotide-directed Splicing: Therapeutic Strategies* critically reviewed current progress in the field and discussed new technical advances in the synthesis and properties of oligonucleotides. (A report of the meeting was published in *Science 322:* 1454–1455 [2008].)

The year 2008 was also the first time that a Banbury Center meeting was not held at Banbury! We had planned a discussion meeting on *Epigenetics: Mechanisms and Regulation*, but the Robertson House renovations meant that there was no time to have it at the Banbury estate. The Meetings and Courses office stepped in to help and arranged for the meeting to be in the Plimpton Room on the main campus. We are very grateful to David Stewart and Val Pakaluk for their hard work done on our behalf. The meaning of the term "epigenetics" has come to cover a very wide range of biological processes, from dynamic short-lived chromatin-mediated gene regulation to long-term alteration of chromatin and other extrachromosomal proteins in nonreplicating cells. Participants were encouraged to think beyond details of molecular mechanisms and to consider how "epigenetics" should be defined. As happens whenever scientists come together to define terms, the discussions proved to be very lively!

For the second year, there has been a significant change in Banbury Center staff. In May 1978, Victor McElheny was appointed as director of the Center. He was followed, in September 1978, by the appointment of Beatrice Toliver as administrative assistant, a position that she has held for 30 years, providing all four Banbury directors with essential support. Bea's knowledge of Banbury and its operations was particularly important to me when I became director following the tragic death of Steve Prentis. With her help, we were able to maintain and expand the program. During the 30 years that Bea has been at Banbury, she has become well known to the thousands of scientists who have participated in Banbury meetings. On their behalf, as well as the Laboratory, we wish Bea the happiest of retirements.

Bea Toliver

As always, the operations of the Banbury Center depend on many people: Ellie Sidorenko at the Conference Room, Basia Polakowski at Robertson House, and Mike Peluso and the grounds crew who look after the Banbury estate. We have many interactions with David Stewart and his staff in the Meetings and Courses office, and, of course, we could not do anything here without the work of the Laboratory's Culinary Services and Housekeeping.

Jan Witkowski
Executive Director

BANBURY CENTER MEETINGS

Genes and the Environment: New Strategies for Research on Multiple Sclerosis

February 3–6

FUNDED BY **Stanley Trotman, Jr. Trust**

ARRANGED BY **B. Greenberg,** The Johns Hopkins Hospital
A. Mellor, Accelerated Cure Project for Multiple Sclerosis
H. Schmidt, Accelerated Cure Project for Multiple Sclerosis

The goal of this meeting was to explore what would be the ideal study for identifying gene–environment interactions involved in multiple sclerosis (MS). Although associations with MS have been identified for a few risk factors such as the HLA-DR2 gene haplotype and cigarette smoke, very little progress has been made in explaining the specific biological role of these factors and the manner in which risk factors interact in the development of MS. However, there are reasons to hope for faster progress in the coming years. High-throughput technologies such as genomic microarrays and new DNA sequencers are producing data in a much more cost-effective fashion than ever before and enabling new experimental strategies. Participants in the meeting were drawn from a wide variety of research areas including MS clinical research, genetics, genomics, environmental toxicology, and epidemiology.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Welcoming Remarks: **B. Greenberg,** The Johns Hopkins Hospital, Baltimore, Maryland: Overview of the agenda/schedule and objectives.

SESSION 1
Chairperson: B. Greenberg, The Johns Hopkins Hospital, Baltimore, Maryland

B. Greenberg, The Johns Hopkins Hospital, Baltimore, Maryland: Overview of multiple sclerosis: Knowns and unknowns.
A. Bar-Or, Montreal Neurological Institute, Canada: Immunology of multiple sclerosis.
H. Schmidt, Accelerated Cure Project for Multiple Sclerosis, Waltham, Massachusetts: Genetics of multiple sclerosis.
S. Subramaniam, Vanderbilt Stallworth Rehabilitation Hospital, Nashville, Tennessee: Role of DNA damage pathway in MS.
W.R. McCombie, Cold Spring Harbor Laboratory: Massively parallel targeted resequencing: Opportunities and challenges.

SESSION 2
Chairperson: A. Ascherio, Harvard School of Public Health, Boston, Massachusetts

S. Jacobson, NINDS/National Institutes of Health, Bethesda, Maryland: Virus associations and MS.
W.I. Lipkin, Columbia University, New York: Identifying viral pathogens in neurological disease.
C. Hayes, University of Wisconsin, Madison: Vitamin D and MS.
B. Winstock-Guttman, SUNY University of Buffalo, New York: Gender and MS.
D. Mohr, Northwestern University, Chicago, Illinois: Stress and MS.
S. Schmidt, Duke Center for Human Genetics, Durham, North Carolina: Gene–environment interaction in age-related macular degeneration: A success story.
B. Banwell, Hospital for Sick Children, Toronto, Canada: Pediatric MS: Insights into the disease.

SESSION 3
Chairperson: T. Vollmer, Barrow Neurological Institute, Phoenix, Arizona

B. Greenberg, The Johns Hopkins Hospital, Baltimore, Maryland: Overview of agenda and goals for the day.
S.D. Vernon, CFIDS Association of America, Charlotte, North Carolina: Empiric delineation of patient heterogeneity to improve diagnosis and intervention.
T. Vollmer, Barrow Neurological Institute, Phoenix, Arizona:

Biorepositories: Challenges and applications.

A. Ascherio, Harvard School of Public Health, Boston, Massachusetts: Use of prospective cohorts in investigating causes of disease.

D.E. Ganem, University of California, San Francisco: Discovering novel pathogens.

SESSION 4

B. Greenberg, The Johns Hopkins Hospital, Baltimore, Maryland

E.W. Daw, Washington University, St. Louis, Missouri: Oligogenic modeling with environmental covariates for association and linkage studies.

D.W. Bigwood, Diogenix, Inc., Madison, Connecticut: Analysis of large gene expression data sets: Strategies for identifying novel pathogenic pathways.

M. Wigler, Cold Spring Harbor Laboratory: How might one apply copy-number analysis to understand the genetics of multiple sclerosis?

Developing a Study Protocol

Discussion initiated by B. Greenberg, The Johns Hopkins Hospital, Baltimore, Maryland: Day conclusions.

SESSION 5

Presentation of Proposed Protocol

B. Greenberg, The Johns Hopkins Hospital, Baltimore, Maryland

Discussion and revision of Protocol.

Banbury Conference Center

GEM Meeting

February 13–14

FUNDED BY **The Stanley Foundation**

ARRANGED BY **S. Gary,** Cold Spring Harbor Laboratory

The Genetics of Early-onset Mania (GEM) Study of Bipolar Disorder is a research project organized by Cold Spring Harbor Laboratory and supported by a donation from Ted and Vada Stanley. This collaborative project involves four institutions: CSHL, Zucker Hillside Hospital–North Shore LIJ, NIMH, and Johns Hopkins University. The goal of this project is to identify genes that may be associated with bipolar disorder by (1) performing genome-wide analysis of copy-number variation in bipolar families, (2) assessing the overall contribution of de novo and inherited mutations in sporadic and familial bipolar disorder, and (3) identifying novel candidate genes for further study.

Participants in the GEM project have initiated a new collection focusing on trios (affected child plus both parents) with the goal of collecting samples from 225 patients and their parents. This Banbury meeting brought together the GEM collaborators to provide a project update and to address the following points: (1) increasing the sample size of collection, (2) the GEM database for entering clinical data, (3) an update on CNV analysis of these and related samples, and (4) the next steps for the project.

Welcome, Opening Remarks: **J.D. Watson,** Cold Spring Harbor Laboratory

Overview and Status of GEM Project: **S. Gary,** Cold Spring Harbor Laboratory

Discussion for Increasing Sample Size (Protocol Changes; Identifying Existing Collections with Ability to Collect Parental DNAs: Other Ideas?)

F. McMahon, E. Leibenluft, T. Schulze, NIMH/National Institutes of Health, Bethesda, Maryland

Group Discussion

Phenotype Discussion/Neurocognition Collection

K. Burdick, Zucker Hillside/LIJ, Glen Oaks, New York

E. Leibenluft, NIMH/National Institutes of Health, Bethesda, Maryland

Presentation about GEM Database/Website

J. Pearl, Data Related, NIMH/National Institutes of Health, Bethesda, Maryland

T. Leotta and V. Makarov, Cold Spring Harbor Laboratory

Update on Recent Results

J. Sebat, Cold Spring Harbor Laboratory

Other Recent Findings in Genetics of Bipolar Disorder

Next Steps (Analysis, Data Sharing, etc.)

Group Discussion

Living on Human Beings: Metagenomic Approaches and Challenges

March 2–5

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **E.F. DeLong,** Massachusetts Institute of Technology
J.I. Gordon, Washington University School of Medicine
G.M. Weinstock, Washington University School of Medicine

Traditional methods of studying microorganisms begin with the isolation of single cells from all those present in a sample, followed by their culture. This introduces the bias that only those organisms that can be cultured can be studied. In contrast, metagenomics is concerned with the characterization of the entire community of microorganisms in a sample. This can be done because new sequencing strategies can sequence gigabases of DNA and bioinformatics strategies can find sequences of interest in these gigabases of sequence. There are projects under way to examine the entire microbiota of environments such as the oceans, soil, and air. Most excitingly, metagenomics offers the possibility of treating the human body as a set of habitats and examining the microbial communities of each habitat, whether the mouth, skin, or gut. This meeting was designed to define and suggest solutions to some of the conceptual and experimental challenges that this field faces.

G. Weinstock, R. Gunsalus

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introduction: Why This Meeting?: **G.M. Weinstock,** Washington University School of Medicine, St. Louis, Missouri

SESSION 1: Approaches for Studying Environmental Communities and Their Operations
Chairperson: B. Birren, Broad Institute, Cambridge, Massachusetts

E.F. DeLong, Massachusetts Institute of Technology, Cambridge: Integrating microbial community genomics and (eco)systems biology: Problems and prospects.
S.W. Chisholm, Massachusetts Institute of Technology, Cambridge: Genome-enabled metagenomics: *Prochlorococcus* as a case study.
M. Polz, Massachusetts Institute of Technology, Cambridge: Identifying ecologically differentiated populations.
A.Z. Worden, Monterey Bay Aquarium Research Institute, Moss Landing, California: Targeted metagenomics of marine eukaryotes.
E.G. Ruby, The University of Wisconsin, Madison, Wisconsin: Comparative genomic analyses of a beneficial bacterial association: Using natural models to ask how mutualisms are maintained.

SESSION 2: Approaches for Characterizing Animal Body Habitat-associated Communities and Their Operations
Chairperson: S. Eddy, Howard Hughes Medical Institute, Ashburn, Virginia

R.L. Hettich, Oak Ridge National Laboratory, Tennessee: A proteogenomic approach for characterizing the molecular activities of gut microbiome.
J. Ravel, University of Maryland School of Medicine, Rockville: Genomic tools for studying the ecology of the human vagina.
H. Flint, Rowett Research Institute, Aberdeen, United Kingdom: Molecular and cultural approaches to functional analysis of the human intestinal microbiota.
J. Segre, National Human Genome Research Institute, Bethesda, Maryland: Survey of skin microflora in healthy volunteers.
M.J. Blaser, New York University School of Medicine, New York: Approaches to defining the cutaneous microbiota in health and disease.
S. Dusko Ehrlich, INRA, Jouy-en-Josas, France: MetaHIT: The European project on metagenomics of human intestinal tract.

SESSION 3: Microbial Evolution, Phylogeny, and Diversity
Chairperson: J. Ravel, University of Maryland School of Medicine, Rockville

N.R. Pace, University of Colorado, Boulder: Molecular microbiology of the human environment.
F.E. Dewhirst, Forsyth Institute, Boston, Massachusetts: The human oral microbiome.
R. Knight, University of Colorado, Boulder: Phylogenetically informed community comparisons using metagenomic data.
C. M. Fraser-Liggett, University of Maryland School of Medicine, Baltimore: Surveying human microbiome diversity: How deep do we go?
R. Gunsalus, University of California, Los Angeles: The essential biology of the anaerobic microbial food chains.

SESSION 4: Technology: What’s Needed to Move Beyond Present Practices
Chairperson: E.F. DeLong, Massachusetts Institute of Technology, Cambridge

J.K. Nicholson, Imperial College London, United Kingdom: The microbiome-mammalian-metabolic axis in health and disease.
B. Birren, Broad Institute, Cambridge, Massachusetts: Genome sequencing using new technologies.
S.C. Schuster, Pennsylvania State University, University Park: Next-generation sequencing and MEGAN metagenomics analysis.
R. Lasken, J. Craig Venter Institute, San Diego, California: High-throughput single-cell genomics pipeline: Applications to the HMB and the hospital environment.
G.M. Weinstock, Washington University School of Medicine, St. Louis, Missouri: The Human Microbiome Project.

SESSION 5: Informatics/Data Analysis: Attaching the Bottlenecks
Chairperson: S.C. Schuster, Pennsylvania State University, University Park

P. Hugenholtz, DOE Joint Genome Institute, Walnut Creek, California: Resolving genetic gradients using fine-scale metagenomics.
S. Eddy, Howard Hughes Medical Institute, Ashburn, Virginia: Advances in large-scale protein sequence analysis: Pfam and HMMER.
A. Godzik, The Burnham Institute, La Jolla, California: Understanding diversity and divergence in (among others) metagenomics data sets.
S.A. Kravitz, J. Craig Venter Institute, Rockville, Maryland: Challenges of large-scale metagenomics data management.

SESSION 6: Meeting Summary and General Discussion
Chairperson: J.I. Gordon, Washington University School of Medicine, St. Louis, Missouri

J. Segre, A. Gardner

Recent Advances and a Multilevel Analysis From FMRP Biology to Clinical Trials

March 9–12

FUNDED BY **NIMH Grant to the University of Illinois**

ARRANGED BY
E. Berry-Kravis, Rush University Medical Center
K. Clapp, FRAXA Research Foundation
W.T. Greenough, University of Illinois
E. Klann, New York University
P.W. Vanderklish, Scripps Research Institute

Significant advances have been made in several areas of fragile X research, particularly those that shed new light on underlying mechanisms. The meeting was designed to review these new findings and to encourage new ideas on the basic relationships between FMRP function, the neurobiological origins of symptoms, and potential treatments. In particular, participants discussed FMRP function and regulation; proteomic and high-specificity FMRP target analyses; alterations in synaptic plasticity, structure, and signaling coupled to mGluR and non-mGluR pathways; mechanistic commonalities between fragile X and other syndromic forms of mental retardation with autism as an endophenotype; systems level approaches to understanding fragile X syndrome and autism; and clinical trials.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Opening Comments: **K. Clapp,** FRAXA Research Foundation, Newburyport, Massachusetts

SESSION 1: Fragile X Phenotypes and Underlying Neural Systems
Chairperson: M.R. Tranfaglia, FRAXA Research Foundation, Newburyport, Massachusetts

R.J. Hagerman, M.I.N.D. Institute, Sacramento, California: Quantitative measures of CNS function for medication trials and comments on ganaxolone study.

D.P. Kennedy, California Technology Institute, Pasadena: Functional abnormalities of the default network in autism and fragile X syndrome.

R. Zorovic, K. Clapp, M. Bear

D.L. Nelson, Baylor College of Medicine, Houston, Texas: Conditional mutations in Fmr1 and Fxrs in the mouse: An update on genotypes and phenotypes.

J.R. Larson, University of Illinois, Chicago: Olfactory learning in the fragile X mouse.

A. El Idrissi, College of Staten Island, New York: Potential pharmacotherapeutic actions of taurine in a mouse model of fragile X syndrome.

R.E. Paylor, Baylor College of Medicine, Houston, Texas: Pharmacological modification of *Fmr1* KO behavioral phenotypes.

I. Ethell

SESSION 2: Synaptic Morphology Phenotypes and Local Network Abnormalities
Chaiperson: W.T. Greenough, University of Illinois, Urbana

W.T. Greenough, University of Illinois, Urbana: Short tutorial: Neurobiology of fragile X—A review.

I. Ethell, University of California, Riverside: Minocycline accelerates dendritic spine maturation and alleviates behavioral defects in animal model.

K. Broadie, Vanderbilt University and Medical School, Nashville, Tennessee: Roles of FMRP in neuronal architecture development and synaptogenesis.

SESSION 3: Distribution, Functions, and Regulation of FMRP
Chairperson: D.L. Nelson, Baylor College of Medicine, Houston, Texas

D. Morris, University of Washington, Seattle: Fmr1 transcript isoforms: Association with polyribosomes; regional and developmental expression in brain.

M. Ramaswami, Trinity College, Dublin, Ireland: Neuronal FMRP particles and their similarities to P bodies.

G.J. Bassell, Emory University, Atlanta, Georgia: Stimulating travels and functions of FMRP in dendrites and axons.

S. Ceman, University of Illinois, Urbana: FMRP expression during earned vocalizations in male zebra finch.

SESSION 4: Proteomics and FMRP mRNA Target Analyses
Chairperson: W.T. Greenough, University of Illinois, Urbana

J. Darnell, The Rockefeller University, New York: Cross-linking Co-IP (CLIP) identification of novel pre- and postsynaptic RNA targets of FMRP.

H. Tiedge, State University of New York, Brooklyn: FMRP and small RNAs.

J.S. Malter, University of Wisconsin, Madison: APP, Abeta, and fragile X syndrome.

S.J. Tapscott, Fred Hutchinson Cancer Research Center, Seattle, Washington: An antisense transcript spanning the CGG repeat region of FMR1 is up-regulated in premutation carriers but silenced in full mutation individuals.

SESSION 5: Modulation of Synaptic Plasticity and Signaling by FMRP
Chairperson: M.F. Bear, Massachusetts Institute of Technology, Cambridge

K.M. Huber, University of Texas Southwestern Medical Center, Dallas: Impaired excitatory drive of neocortical inhibitory neurons may contribute to longer persistent activity states in Fmr1 KO mice.

J. Lauterborn, University of California, Irvine: Hippocampal LTP deficits in fragile X: Restoration of synaptic plasticity by BNDF.

A. Bhattacharyya, University of Wisconsin, Madison: cAMP signaling in FX brain.

M.C. McKenna, University of Maryland School of Medicine, Baltimore: Altered neuronal and astrocytic glutamate metabolism in 18-day-old *Fmr 1* knockout mouse brain: Normalization by MPEP.

R.S. Zukin, Albert Einstein College of Medicine, Bronx, New York: Dysregulation of mTOR signaling in mouse model of fragile X syndrome.

S.T. Warren, Emory University School of Medicine, Atlanta, Georgia: FMRP signaling pathway mediated by phosphorylation.

I.J. Weiler, University of Illinois, Urbana-Champaign: Aberrant phosphatase activation in fragile X syndrome.

SESSION 6: Mechanistic Parallels between FXS and Other Neuropsychiatric Conditions
Chairperson: E. Klann, New York University, New York

E. Klann, New York University, New York: Short tutorial: Altered translational control in fragile X model mice and other mouse models of MR and ASD.

M.F. Bear, Massachusetts Institute of Technology, Cambridge: Studies of protein synthesis in hippocampus.

M.A. Smith, Case Western Reserve University, Cleveland, Ohio: Parallels between fragile X and Alzheimer's disease.

SESSION 7: Progress in Clinical Development
Chairperson: S.A. Warren, Emory University School of Medicine, Atlanta, Georgia

E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois: Update on lithium treatment in fragile X syndrome.

B.A. Oostra, Erasmus Universiteit Rotterdam, The Netherlands: The effect of mGluR5 antagonists in vitro and in vivo.

C. Erickson, Riley Hospital for Children, Indianapolis, Indiana: Aripiprazole in fragile X patients.

F. Gasparini, Novartis Pharma AG, Basel, Switzerland: Targeting Group I and II metabotropic glutamate receptors: Drug discovery and potential therapeutic indications.

Algebraic Statistics, Machine Learning, and Lattice Spin Models

March 16–19

FUNDED BY **Clay Mathematics Institute**

ARRANGED BY **J. Carlson,** Clay Mathematics Institute
D.A. Ellwood, Clay Mathematics Institute
P.P. Mitra, Cold Spring Harbor Laboratory

P. Mitra, R. Shrock

Recently, there have been exciting advances in the application of ideas and algorithms from commutative algebra and group theory to problems of data analysis and statistics, particularly in computational genomics. However, these ideas are not yet widely known to other communities of theorists who may benefit from these developments. The goal of this workshop was to bring together mathematicians working in algebraic statistics with researchers in machine learning and statistical physics for mutual pedagogy and for exploration of new research avenues opened up by the application of algebraic techniques to data.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

P. Parrilo, B. Mishra

SESSION 1
Chairperson: C. Myers, Cornell University, Ithaca, New York

P.A. Parrilo, Massachusetts Institute of Technology, Cambridge: Semigroups and semidefinite programming.
R. Rabadan, Institute for Advanced Study, Princeton, New Jersey: Viruses.

SESSION 2
Chairperson: P.P. Mitra, Cold Spring Harbor Laboratory

L. Pachter, University of California, Berkeley, and S. Sullivan, Harvard University, Cambridge, Massachusetts: Algebraic statistics tutorial.
L. Pachter, University of California, Berkeley, and S. Sullivan, Harvard University, Cambridge, Massachusetts: Algebraic statistics tutorial (cont'd.).

Informal Discussions

SESSION 3
Chairperson: A. Sengupta, Rutgers, The State University of New Jersey

B. Mishra, New York University, New York: Zero-one phenomena in genome sequencing.

SESSION 4
Chairperson: D. Huse, Princeton University, New Jersey

G. Carlsson, Stanford University, California: Algebraic topology for data analysis.
C. Sire, Université Paul Sabatier, Toulouse, France: Poker and statistical physics.

How Will We Be Able to Cure Most Cancers?

March 30–April 2

FUNDED BY **OSI Pharmaceuticals, Inc.**

ARRANGED BY
A.J. Levine, Institute of Advanced Studies
C.L. Sawyers, Memorial Sloan-Kettering Cancer Center
R.A. Weinberg, Whitehead Institute for Biomedical Research
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

We have been performing research on cancer for more than a century and, since the early 1970s, we have discovered many of the genetic and biochemical changes that turn a normal cell into a cancer cell. Furthermore, our knowledge of the fundamental biology of cancer is likely to undergo another major increase as genome-based techniques begin to be used on a massive scale to characterize the full set of molecular changes in many tumor types. However, the number of cancers that can at present be cured effectively and efficiently remains small. Why is this? Do cancers vary so much that a treatment specific to one patient's cancer may be ineffective for another patient? What targets are currently available for drug therapy? What can be done to increase the number? What is the potential for combination therapies? What can be done to improve early detection? May we have to face the fact that many cancers may not be curable?

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Remarks: **J.D. Watson,** Cold Spring Harbor Laboratory

Introduction: The Problem: M.J. Thun, American Cancer Society, Atlanta, Georgia: Cancer trends.

SESSION 1
Chairperson: R. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

C. Lengauer, Novartis Institutes for Biomedical Research, Inc., Cambridge, Massachusetts: Oncology target validation—taken seriously.
J. Barsoum, Synta Pharmaceuticals Corporation, Lexington, Massachusetts: Advancing Elesclomol from the lab to phase 3: Targeting cancer by the selective induction of oxidative stress.
R. Cohen, Genentech, South San Francisco, California: Targeted therapies: Lessons from Herceptin.
Y. Luo, Tsinghua University, Beijing, China: Molecular cancer therapy of endostatin: The end of the beginning.

SESSION 2
Chairperson: B. Stillman, Cold Spring Harbor Laboratory

D. Reinberg, New York University School of Medicine, New York: Histone deacetylases, methylases and demethylases: Current status, future potentials.
J. Schlessinger, Yale University School of Medicine, New Haven, Connecticut: Developing new therapies: The example of PLX4720 and B-RafV600E.
J. Peto, London School of Hygiene & Tropical Medicine, London, United Kingdom: The costs and benefits of HPV vaccination.
M. Stratton, The Sanger Institute, Cambridge, United Kingdom: Patterns of somatic mutation in human cancer genomes.
J.B. Hicks, Cold Spring Harbor Laboratory: Breast tumor architecture and progression analyzed by genomic method.
B. Weber, GlaxoSmithKline, Collegeville, Pennsylvania: Genomic approach to targeted drug development.

SESSION 3
Chairperson: B. Stillman, Cold Spring Harbor Laboratory

R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Mechanisms of malignant progression.
P.A. Beachy, Stanford University, California: Hedgehog signaling and cancer.
T. Curran, Children's Hospital of Philadelphia, Pennsylvania: Targeting the Sonic Hedgehog pathway in pediatric brain tumors: Promise and problems.
L.C. Cantley, Harvard Medical School/Beth Israel Deaconess Medical Center, Boston, Massachusetts: Targeting the PI3K pathway.
A.J. Levine, Institute for Advanced Study, Princeton, New Jersey: New methods for identifying drug targets.
C.B. Harley, Geron Corporation, Menlo Park, California: Telomerase-based therapies: Potential to hit a stem cell target.

SESSION 4
Chairperson: B. Clarkson, Memorial Sloan-Kettering Cancer Center, New York

P.-P. Pandolfi, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Biochemical and genetic pathways as sources of targets.
G. Evan, University of California, San Francisco: Targeting the untargetable—Modeling anti-Myc therapy in mouse cancer models.
S. Lowe, Cold Spring Harbor Laboratory: Mouse models in cancer gene discovery and cancer therapy.
C.J. Sherr, St. Jude Children's Research Hospital, Memphis, Tennessee: Why BCR-ABL-induced acute lymphoblastic leukemia (Ph^+ ALL) responds poorly to targeted therapy.
A. Ashworth, Institute of Cancer Research, London, United Kingdom: Synthetic lethal approaches to cancer therapy.
P. Pharoah, Strangeways Research Laboratory, Cambridge, United Kingdom: Polygenic susceptibility in breast cancer.

SESSION 5
Chairperson: R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

G. Dranoff, Dana-Farber Cancer Institute, Boston, Massachusetts: Balancing tumor immunity and inflammatory pathology.
D.M. Epstein, OSI Pharmaceuticals, Inc., Farmingdale, New York: Evidence for pathological epithelial to mesenchymal transition and an alternate rationale for multitargeting in cancer.
R. Kalluri, Harvard Medical School, Boston, Massachusetts: Tumor microenvironment controls the rate of cancer progression and metastasis.
P. Dirks, Hospital for Sick Children, Toronto, Canada: Cancer stem cells as therapeutic targets.
G.F. Vande Woude, Van Andel Institute, Grand Rapids, Michigan: Complexities and challenges: Met and malignant progression.

SESSION 6
General Discussion: What Next?

B. Clarkson, J. Watson, J. Schlesinger, R. Weinberg

Molecular Mechanisms Modulating Skeletal Muscle Mass and Function

April 6–9

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **A.L. Goldberg,** Harvard Medical School
D.J. Glass, Novartis Institutes for Biomedical Research

Many conferences have focused on the early development of skeletal muscle, the roles of satellite cells, or contractile mechanisms; however, this conference reviewed the mechanisms for muscle homeostasis in the adult animal and human. As anyone who has had a limb immobilized will appreciate, the loss of muscle can be rapid and very hard to replace. Participants in the meeting considered questions such as What are the molecular mechanisms that occur in response to increased exercise that result in hypertrophy and/or fiber-type switching, and how does inactivity lead to fiber atrophy? How are protein synthesis, proteolysis, and gene expression altered in skeletal muscle during the wasting (cachexia) induced by cancer, cardiac failure, sepsis, and renal failure? How do cytokines and hormones influence the properties of muscle in normal and disease states?

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introduction: Why this meeting?: **A.L. Goldberg,** Harvard Medical School, Boston, Massachusetts

SESSION 1: Skeletal Muscle Development and Differentiation
Chairpersons: S.-J. Lee, Johns Hopkins University School of Medicine, Baltimore, Maryland, and **G. Pavlath,** Emory University School of Medicine, Atlanta, Georgia

R. Krauss, Mount Sinai School of Medicine, New York: Role of the Ig superfamily receptors CDo and neogenin in myogenesis.
M.A. Rudnicki, Ottawa Health Research Institute, Canada: Molecular mechanisms regulating satellite cell function.
A. Wagers, Harvard Medical School, Boston, Massachusetts: Regenerative potential of skeletal muscle stem cells.
G. Pavlath, Emory University School of Medicine, Atlanta, Georgia: Molecular control of satellite cell function during muscle growth.
K.A. Esser, University of Kentucky College of Medicine, Lexington: Clock genes and adult skeletal muscle structure and function.

SESSION 2: Muscle Disease and Dystrophy
Chairpersons: N. Rosenthal, European Molecular Biology Laboratory, Monterotondo, Italy, and **K.P. Campbell,** University of Iowa College of Medicine, Iowa City

M.R. Capecchi, University of Utah, Salt Lake City: Role of muscle lineage in muscle malignancy.
D.C. Guttridge, Ohio State University, Columbus: Muscle wasting in cancer cachexia and lessons learned from muscular dystrophy.
K.P. Campbell, University of Iowa College of Medicine, Iowa City: Muscular dystrophy as a complex disease: Insights from mouse models.
P. Munoz-Canoves, Centre for Genomic Regulation, Barcelona, Spain: Cytokine-mediated skeletal muscle hypertrophy.
N. Rosenthal, European Molecular Biology Laboratory, Monterotondo, Italy: Enhancing muscle regeneration.
Maryland: Funding.

N. Rosenthal

SESSION 3: Ubiquitin-dependent Protein Breakdown in Muscle
Chairperson: A.L. Goldberg, Harvard Medical School, Boston, Massachusetts

D.J. Glass, Novartis Institutes for Biomedical Research, Cambridge, Massachusetts: Signaling pathways that mediate skeletal muscle atrophy and hypertrophy.
D. Attaix, Human Nutrition Research Center of Clermont-Ferrand, Ceyrat, France: Identification of polyubiquitinated substrates of the muscle proteasome.
M. Gautel, King's College, London, United Kingdom: Sarcomeric links to ubiquitination and autophagy pathways.
S. Wing, McGill University, Montreal, Canada: Role of the USP19 deubiquitinating enzyme in muscle cell proliferation.
C. Patterson, North Carolina University, Chapel Hill: Multiple roles of ubiquitin ligases in muscle biology.
G. Nuckolls, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland: Funding.

SESSION 4: Regulation of Protein Breakdown and Synthesis in Muscle
Chairperson: D.J. Glass, Novartis Institutes for Biomedical Research, Cambridge, Massachusetts

A.L. Goldberg, Harvard Medical School, Boston, Massachusetts: Contributions of the autophagic and ubiquitin proteasome pathways to muscle atrophy.
M.F. Sandri, Dulbecco Telethon Institute at Venetian, Padova, Italy: Regulation of proteolytic systems during muscle wasting.
M. Spencer, University of California Los Angeles: Proteolysis and regulation of muscle mass by calpain 3.
S. Schiaffino, Venetian Institute of Molecular Medicine, Padova, Italy: Activity-dependent signaling pathways controlling muscle fiber size and type.
M.A. Ruegg, University of Basel, Switzerland: Role of the mTOR complex 1 (mTORC1) and mTORC2 in skeletal muscle.
P. Puigserver, Harvard Medical School, Boston, Massachusetts: Transcriptional mechanism modulating mitochondrial oxidative skeletal muscle function.

SESSION 5: Growth Factors and Therapeutic Challenges
Chairpersons: S. Schiaffino, Venetian Institute of Molecular Medicine, Padova, Italy, and **L.A. Leinwand,** University of Colorado, Boulder

S.-J. Lee, Johns Hopkins University School of Medicine, Baltimore, Maryland: Regulation of muscle growth by myostatin.
K. Wagner, The Johns Hopkins Hospital, Baltimore, Maryland: Clinical considerations for modulators of muscle growth.
D. Clemmons, University of North Carolina, Chapel Hill: IGF-1 and muscle cell growth and differentiation.
L.A. Leinwand, University of Colorado, Boulder: Exercise, diet, and gender effects on skeletal muscle.
W.E. Mitch, Baylor College of Medicine, Houston, Texas: Mechanisms elicited by kidney disease to cause muscle protein losses.
K. Walsh, Boston University School of Medicine, Massachusetts: Akt-mediated growth of type IIb fibers reduces fat mass and improves metabolic parameters in obese mice.

SESSION 6: Meeting Summary and General Discussion
Chairperson: D.J. Glass, Novartis Institutes for Biomedical Research, Cambridge, Massachusetts

M. Capecchi, M. Rudnicki

W. Mitch, D. Attaix

Theoretical and Experimental Approaches to Auditory and Visual Attention

April 20–23

FUNDED BY **The Swartz Foundation**

ARRANGED BY **H. Cohen,** The Swartz Foundation
J.B. Fritz, University of Maryland
J.H. Reynolds, The Salk Institute For Biological Studies

Research in the human and macaque has provided a wealth of information on the neural mechanisms that mediate visual attention. Recent psychoacoustic and neurophysiological studies of attention in the auditory system, and research on interactions of visual and auditory attention, have added considerably to this picture. These studies have found parallels with visual attention mechanisms but have also raised new questions, such as the role of adaptive plastic changes in spectrotemporal receptive field shape during selective attention and the nature of the coordination of attention-driven changes at multiple processing levels from cochlea to cortex. The inherently temporal nature of auditory stimuli has also led to interesting insights into the temporal dynamics of auditory attention. The purpose of this workshop was to bring together experimentalists and theoreticians working in auditory and visual attention for a vibrant discussion of current research.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
J.H. Reynolds, The Salk Institute For Biological Studies, La Jolla, California, and
J.B. Fritz, University of Maryland, College Park

SESSION 1: Experimental and Theoretical Perspectives on Attention
Chairperson: R. Desimone, Massachusetts Institute of Technology, Cambridge

K. Nakayama, Harvard University, Cambridge, Massachusetts: Perception, cognition, and action.

S.A. Hillyard, University of California, San Diego, La Jolla: Attention facilitates multiple features in parallel in human visual cortex.

J. Duncan, MRC Cognition & Brain Sciences Unit, Cambridge, United Kingdom: Selective behavior and selective attention in the human and monkey brain.

R.H. Wurtz, National Eye Institute, National Institutes of Health, Bethesda, Maryland: Visual gateway to cortex and its guardian attention in the LGN and TRN.

M. Carrasco, New York University, New York: Effects of spatial and feature-based attention: Psychophysical and neuroimaging studies.

SESSION 2: Experimental and Theoretical Perspectives on Attention (cont'd.)
Chairperson: L. Itti, University of Southern California, Los Angeles

L.F. Abbott, Columbia University, New York: Gating of multiple signals through attentional modulation.
D. Heeger, New York University, New York: The normalization model of attention.

SESSION 3: Auditory Attention: Human
Chairperson: S.A. Shamma, University of Maryland, College Park

R.J. Zatorre, McGill University, Montreal, Canada: Functional organization of human auditory cortex: Bottom-up features and top-down processes.
E. Hafter, University of California, Berkeley: A role for memory in shared attention.
R. Carlyon, MRC Cognition & Brain Sciences, Cambridge, United Kingdom: Effects of attention on auditory scene analysis.
B. Shinn-Cunningham, Boston University, Massachusetts: The costs of switching auditory attention.
C. Alain, Rotman Research Institute of Baycrest Centre, Ontario, Canada: Top-down influences on memory and response-related activity for sound location (dual pathways, parietal cortex, and spatial memory).

SESSION 4: Visual Attention I: Theory and Experiment
Chairperson: R.H. Wurtz, National Eye Institute, National Institutes of Health, Bethesda, Maryland

S. Treue, German Primate Center, Goettingen, Germany: Spatial, feature, and object-based attention in area MT.
L. Itti, University of Southern California, Los Angeles: Quantifying bottom-up and top-down influences on gaze allocation in humans and monkeys.
L. Chelazzi, University of Verona Medical School, Italy: Mechanisms of feature-selective attention in area V4 of the macaque (task relevance of responses in V4).

SESSION 5: Auditory Attention I: Neurophysiology of Auditory Attention
Chairperson: R. Carlyon, MRC Cognition & Brain Sciences, Cambridge, United Kingdom

T. Zador, Cold Spring Harbor Laboratory: Two components of attentional modulation in rat auditory cortex.
S.A. Shamma, University of Maryland, College Park: Attention and rapid plasticity in auditory cortex.
J.B. Fritz, University of Maryland, College Park: What is the contribution of frontal cortex to an auditory attentional network?

SESSION 6: Visual Attention II
Chairperson: K. Nakayama, Harvard University, Cambridge, Massachusetts

W.S. Geisler, University of Texas at Austin: Mechanisms of fixation selection evaluated using ideal observer analysis.
J.C. Martinez Trujillo, McGill University, Montreal, Canada: Attentional modulation of sensory inputs at the level of single neurons in MT.
P. Cavanagh, Harvard University, Cambridge, Massachusetts: Object-based integration and moving attention.

SESSION 7: Cell Type Specificity
Chairperson: L. Chelazzi, University of Verona Medical School, Italy

J.H. Reynolds, The Salk Institute for Biological Studies, La Jolla, California: Mapping the microcircuitry of attention.
J. Mitchell, The Salk Institute, San Diego, California: Attention-dependent response modulation varies between cell classes in macaque V4.
X.-J. Wang, Yale University School of Medicine, New Haven, Connecticut: Stochastic and synchronous neural circuit dynamics underlying attentional gain modulation.

SESSION 8: Visual Attention III: Attentional Control
Chairperson: J. Duncan, MRC Cognition & Brain Sciences Unit, Cambridge, United Kingdom

J. Gottlieb, Columbia University, New York: Attention, motor planning, and decisions: The perspective from the parietal cortex.
S. Ganguli, University of California, San Francisco: 1-dim dynamics of attention and decision making in LIP.
J. Mazer, Yale School of Medicine, New Haven, Connecticut: Cortical representations of attention and salience.

SESSION 9: Synchrony and Attention
Chairperson: S. Treue, German Primate Center, Goettingen, Germany

R. Desimone, Massachusetts Institute of Technology, Cambridge: Neural synchrony and selective attention.
E. Neibur, Johns Hopkins University, Baltimore, Maryland: Synchrony and the attentional state.
P. Tiesinga, University of North Carolina, Chapel Hill: Role of interneuron diversity in the cortical circuit for attention.
J. Swartz, The Swartz Foundation, East Setauket, New York: Closing remarks

To What Age Should We Be Expected to Work?

April 27–30

FUNDED BY **Oliver Grace Professorship Fund**

ARRANGED BY **R.N. Butler,** International Longevity Center
M.D. Hurd, Center for the Study of Aging, RAND Corporation
T.B.L. Kirkwood, Newcastle University
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Biomedical research on aging is having an impact on two rather different areas that are not yet closely linked. On the one hand, considerable progress has been made in elucidating some of the genetic changes and molecular processes that contribute to aging in experimental organisms such as *C. elegans,* the fruit fly, and mice. These processes can be manipulated, prolonging the life span of these organisms. On the other hand, our lives are being extended through better lifestyles and improved health care, and thus we are living longer; however, it seems that our maximum life spans are unchanged. Is extending the human life span an achievable or desirable goal? Should we concentrate instead on maintaining the quality of life of the extra years that we are now gaining? That is, can we live longer and still be healthy?

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Why a meeting on this topic?: **J.D. Watson,** Cold Spring Harbor Laboratory

SESSION 1
Chairperson: M.D. Hurd, Center for the Study of Aging, RAND, Santa Monica, California

T.B.L. Kirkwood, Newcastle University, Newcastle upon Tyne, United Kingdom: So why does aging occur?
R.N. Butler, International Longevity Center, New York: Changing patterns of morbidity and mortality across the life course.
V.A. Bohr, National Institute on Aging, Baltimore, Maryland: Genome maintenance and DNA repair, changes with aging.
J.W. Shay, University of Texas Southwestern Medical Center, Dallas: Role of telomerase in aging and cancer.

SESSION 2

Chairperson: J.W. Shay, University of Texas Southwestern Medical Center, Dallas

D.C. Wallace, University of California, Irvine: Mitochondria and the pathophysiology of aging.

S.N. Austad, University of Texas Health Science Center at San Antonio: Comparative mechanisms of aging: An update.

J.M. Ordovas, Tufts University, Boston, Massachusetts: Gene–environment interactions modulating the risk of age-related disorders.

J.A. Faulkner, University of Michigan, Ann Arbor: Age-related changes in skeletal muscles from whole muscles to single fibers.

J. Watson, M. Raff

SESSION 3

Chairperson: T.B.L. Kirkwood, Newcastle General Hospital, Newcastle upon Tyne, United Kingdom

T.E. Johnson, University of Colorado, Boulder: Role of stress in specifying longevity and rate of aging.

L. Hayflick, University of California, San Francisco, The sea ranch: Manipulating the four aspects of the finitude of life.

B.N. Ames, Children's Hospital, Oakland, California: Delaying (or accelerating) the degenerative diseases.

C.B. Harley, Geron Corporation, Menlo Park, California: Telomerase-based therapies: Potential to hit a stem cell target.

M. Harman, Kronos Longevity Research Institute, Phoenix, Arizona: Hormonal changes and hormone replacement in older persons: Promise, hype, and realities.

SESSION 4

Chairperson: D.C. Wallace, University of California, Irvine

N. Barzilai, Albert Einstein College of Medicine, Bronx, New York: Strategies to prevent age-related diseases through human genetics.

T.A. Salthouse, University of Virginia, Charlottesville: Implications of age differences in cognitive functioning for work.

R. Willis, University of Michigan, Ann Arbor: Cognitive capital and the future of work.

H. Fillit, The Alzheimer's Drug Discovery Foundation, New York: Clinical implications of cognitive aging.

T.T. Perls, Boston Medical Center, Massachusetts: The centenarian and supercentenarian looking glass.

D.R. Weir, University of Michigan, Ann Arbor: Does health limit work life?

L. Hayflick, J. Witkowski

SESSION 5

Chairperson: R.N. Butler, International Longevity Center, New York, New York

R. Sutch, University of California, Riverside: Working at advanced ages. Historical evidence and economic perspectives.

K. Christensen, University of Southern Denmark, Odense: Does extreme longevity lead to extreme levels of disability?

K. McGarry, Dartmouth College, Hanover, New Hampshire: Preferences and selection in insurance markets: Evidence from long-term care insurance.

M.D. Hurd, Center for the Study of Aging, RAND Corporation, Santa Monica, California: Demographics of aging around the world.

General Discussion: Aging in the 21st century.

L. Lederman

The Architectural Logic of Mammalian CNS

May 4–7

FUNDED BY **The William M. Keck Foundation, National Science Foundation Grant**

ARRANGED BY **P.P. Mitra,** Cold Spring Harbor Laboratory
L.W. Swanson, University of Southern California

This meeting was held to assess the progress of the Brain Architecture Project. The goals of the Project are to curate human neuroanatomical connectivity information from the existing literature into a knowledge base and to build suitable user and web interfaces. To complement these literature curation efforts, and to help shape the corresponding knowledge base schemas and geometrical templates for the user interface, it would be of great benefit to have an "outline" version of the connectivity diagram of the mammalian brain as well as a list of "rules" that the circuitry has been observed to follow. Participants were asked to present their overview of the architectural logic of the mammalian CNS. These might take the form of circuit diagrams at a coarse level for the full system, more elaborate circuit diagrams for subsystems or substructures that have sufficient generality across species or across brain regions, or specific rules. In contrast with the more familiar morphological approaches to comparative neuroanatomy, the meeting was concerned with the logic of the "highway map."

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Review of Brain Architecture Project
Chairperson: P.P. Mitra, Cold Spring Harbor Laboratory

P.P. Mitra, Cold Spring Harbor Laboratory: Introduction to the Brain Architecture Project and to the meeting theme.

J. Bohland, Cold Spring Harbor Laboratory: Progress and future challenges for the Brain Architecture Project.

H. Bokil, Cold Spring Harbor Laboratory: Analysis of spatial gene expression patterns in the Allen Brain Atlas.

J. Lin, Cold Spring Harbor Laboratory: A literature mining and curation system for the Brain Architecture Project.

SESSION 2: Introduction to Architectural Logic Problem
Chairperson: H. Breiter, Massachusetts General Hospital, Charlestown

L.W. Swanson, University of Southern California, Los Angeles: Understanding the basic wiring diagram of the nervous system.
H.J. Karten, University of California, San Diego, La Jolla: Conservation of microcircuitry across vertebrate phylogeny.
R. Kotter, Radboud University, Nijmegen, The Netherlands: CoCoMac database interfaces for integrating primate brain connectivity.

SESSION 3: Architectural Logic Problem (cont'd.)
Chairperson: A. Graybiel, Massachusetts Institute of Technology, Cambridge

C.B. Saper, Beth Israel Deaconess Medical Center, Boston, Massachusetts: The flip-flop switch as a motif in brain architecture.
J.L. Price, Washington University, St. Louis, Missouri: Hierarchical organization of systems for homeostasis and maintenance of the self.
D. Kleinfeld, University of California, San Diego: (Near) somatic shunting as a circuit motif: Evidence from vision, somatosensation, and epilepsy.
C.C. Hilgetag, Jacobs University Bremen, Germany: Relating cortical connections to cortical architecture.
H. Barbas, Boston University, Massachusetts: Laminar-specific prefrontal pathways for excitatory and inhibitory control.

SESSION 4: Architectural Logic Problem (cont'd.)
Chairperson: M. Hawrylycz, Allen Institute For Brain Science, Seattle, Washington

S. Haber, University of Rochester, New York: Three-dimensional models of fiber tracts arising from specific cortical areas through the primate brain: Pathways to understanding human functional circuitry.
D.C. Van Essen, Washington University School of Medicine, St. Louis, Missouri: Cortical areas, hierarchies, and networks in monkeys and humans.

Discussion of Architectural Logic Problem

P.P. Mitra, Cold Spring Harbor Laboratory: White paper; strategizing for the large-scale connectivity project.

SESSION 5: Architectural Logic (cont'd.)
Chairperson: P. Freed, Columbia University, New York

J.D. Schmahmann, Massachusetts General Hospital, Boston: Principles of organization of cerebral white matter pathways: Implications for the architecture and connections of cortical and subcortical nodes of distributed neural circuits.
N. Makris, Massachusetts General Hospital, Charlestown, and H. Breiter, Massachusetts General Hospital, Charlestown: Methodological and logical challenges in scaling between circuits and function.

SESSION 6: Clinical Perspectives on Brain Architecture
Chairperson: P. Freed, Columbia University, New York

D.G. Herrera, Weill Medical College of Cornell University, New York: Clinical implications of a novel understanding of brain architecture.
J. Safdieh, Weill Medical College of Cornell University, New York: Circuitry in the classroom and the clinic.

J. Schmahmann, H. Breiter

J. Safdieh, P. Freed

Prion Strains: Origins, Mechanisms, and Implications for Disease

May 11–14

FUNDED BY **NIAID and the Medical Research Council, United Kingdom**

ARRANGED BY
B. Caughey, NIAID Rocky Mountain Laboratories
J. Collinge, University College London
C. Soto, University of Texas Medical Branch
C. Weissmann, Scripps Florida

Multiple distinct strains of naturally occurring sheep scrapie can be passaged in mice. Such strains are classically distinguished by their biological properties: They produce distinct incubation periods and patterns of neuropathology in inbred lines of laboratory mice. Furthermore, strains can be reisolated in mice after passage in intermediate species with different PrP primary structures. The widely accepted protein-only hypothesis, if correct, must be able to explain how a single polypeptide chain could encode multiple disease phenotypes. Clearly, understanding how a protein-only infectious agent could encode such phenotypic information is of wide biological interest and raises intriguing evolutionary questions. Do other proteins behave in this way? The novel pathogenic mechanisms involved in prion propagation may be of far wider significance and relevant to other neurological and nonneurological illnesses.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Strains: Definition, Concepts
Chairperson: J. Collinge, University College London, United Kingdom

J. Collinge, University College London, United Kingdom: Prion strains, transmission barriers, and neurotoxicity.

SESSION 2: Strain Typing: Biochemical
Chairperson: J. Collinge, University College London, United Kingdom

M.H. Groschup, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany: Strain typing in animal TSE cases: Criteria for strain definitions.
J. Wadsworth, University College London, London, United Kingdom: Problems in defining human prion strains.
A. Hill, University of Melbourne, Australia: Role of PrP post-translational modifications as markers of prion strain type.
P. Gambetti, Case Western Reserve University, Cleveland, Ohio: Prion strains in human prion diseases.

SESSION 3: Strain Typing In Vivo and in Cell Culture
Chairperson: B. Caughey, NIAID Rocky Mountain Laboratories, Hamilton, Montana

G. Telling, University of Kentucky, Lexington: Transgenic analysis of CWD strains.
U. Agrimi, Instituto Superiore di Sanita, Rome, Italy: Strain typing of animal prions in natural hosts and by transmission to bank voles (*Myodes glareolus*).
H. Laude, Institut National de la Recherche Agronomique, Jouy-en-Josas, France: Transmission of ruminant TSE to transgenic mice: Further insight about prion strain diversity in naturally infected hosts.
G. Zanusso, University of Verona, Italy: Intraspecies transmission of bovine amyloidotic spongiform encephalopathy.

SESSION 4: Structural Basis of Strain-ness
Chairperson: B. Caughey, NIAID Rocky Mountain Laboratories, Hamilton, Montana

W.K. Surewicz, Case Western Reserve University, Cleveland, Ohio: Recombinant prion protein amyloid: Molecular structure, strains, and infectivity.
I. Baskakov, University of Maryland Biotechnology Institute, Baltimore: Generating multiple strains of amyloid fibrils from a single polypeptide chain.
D.S. Eisenberg, University of California, Los Angeles: Structural studies of amyloids, prions, and strains.
R.B. Wickner, NIDDK/NIH, Bethesda, Maryland: Yeast prion amyloid structure explains heritability of “strain” information.

SESSION 5: Structural Basis of Strain-ness (cont’d.)
Chairperson: S.B. Prusiner, University of California, San Francisco

B. Caughey, NIAID Rocky Mountain Laboratories, Hamilton, Montana:
Ultrastructure and strain comparison of underglycosylated, anchorless scrapie prion protein fibrils.

SESSION 6: Strain Biology In Vivo
Chairperson: S.B. Prusiner, University of California, San Francisco

B.W. Chesebro, NIAID, Rocky Mountain Laboratories, Hamilton, Montana: Role of anchorless prion protein in pathogenesis induced by different scrapie strains.
I. Vorberg, Technical University of Munich, Germany: Yeast prion aggregation propensities in mammalian cells.
J.C. Bartz, Creighton University, Omaha, Nebraska: Prion strain interference.
D. Westaway, University of Alberta, Edmonton, Canada: The PrP-like Shadoo protein: Misfolding and in vivo variants.
M. Jeffrey, Veterinary Laboratories Agency, Midlothian, United Kingdom: Species, strain, and cell-associated changes in abnormal PrP processing following prion infection.

SESSION 7: Strain Biology In Vivo (cont’d.)
Chairperson: C. Soto, University of Texas Medical Branch, Galveston

J. Manson, Roslin Institute, Edinburgh, United Kingdom: Prion strains and host susceptibility.
S. Priola, Rocky Mountain Laboratories, NIAID/NIH, Hamilton, Montana: Factors influencing in vitro and in vivo scrapie strain phenotypes.
C. Weissmann, Scripps Florida, Jupiter: Do PrP-linked glycans contribute to prion strain determination?
J.G. Safar, University of California, San Francisco: Conformational intermediates, clearance, and species barrier of natural prion strains.

SESSION 8: Strain Generation and Propagation In Vitro
Chairperson: C. Soto, University of Texas Medical Branch, Galveston

S.B. Prusiner, University of California, San Francisco: Protease-sensitive and -resistant strains of synthetic prions.
C. Soto, University of Texas Medical Branch, Galveston: Generation of multiple new prion strains by in vitro PrP replication.
J. Castilla, Scripps Florida, Jupiter: In vitro studies of the transmission barrier.
S.W. Liebman, University of Illinois at Chicago: The birth of a foreign prion in yeast.

SESSION 9: Varia
Chairperson: S.B. Prusiner, University of California, San Francisco

R.A. Bessen, Montana State University, Bozeman: Using lymphoreticular system replication-deficient prion strains to determine routes of prion neuroinvasion.

SESSION 10: General Discussion and Concluding Remarks
Chairperson: S.B. Prusiner, University of California, San Francisco

S. Liebman

D. Westaway, S. Prusiner, I. Baskakov

Identifying KRAS-targeted Therapeutic Approaches for Pancreatic Cancer

June 16–17

FUNDED BY	**The Lustgarten Foundation for Pancreatic Cancer Research**

ARRANGED BY	**C.J. Der,** University of North Carolina, Chapel Hill **N.K. Tonks,** Cold Spring Harbor Laboratory
ADDITIONAL ORGANIZERS	**K.A. Johnke,** The Lustgarten Foundation for Pancreatic Cancer Research **R. Hruban,** Johns Hopkins Medical Institutions

KRAS mutations occur in 100% of pancreatic cancers, and this meeting focused on targeting the KRAS oncogene for novel therapeutics for pancreatic cancer treatment. Unfortunately, small GTPases such as Ras are not classically considered "druggable" targets, and earlier anti-Ras approaches have not been successful. Nevertheless, there is no doubt that anti-Ras therapeutics have huge potential in the treatment of pancreatic cancer. Two key uncertainties in such efforts are (1) what technologies will be most suitable for functional screens to identify targets for therapeutic intervention and (2) what model of cell/mouse systems should be used to apply these technologies? It is the goal of this meeting to identify the best technologies and systems, so that it will be possible to establish and apply genome-wide screens to identify novel modulators of KRAS-mediated pancreatic cancer growth.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

I. R.F. Vizza, The Lustgarten Foundation, Bethpage, New York: Welcome and introduction
II. R. Hruban, Johns Hopkins Medical Institutions, Baltimore, Maryland: Goals and desired outcome of meeting
III. R. Hruban, Johns Hopkins Medical Institutions, Baltimore, Maryland: Overview of ongoing Lustgarten initiatives
IV. Model Systems
Moderator: C.J. Der, University of North Carolina, Chapel Hill

- Human model cell systems (hTERT-immortalized normal cells)
- Established pancreatic tumor cell lines
- Primary pancreatic tumor isolates
- Pancreatic tumor stem cells
- Mouse models of pancreatic cancer
- Mouse-model-derived cell cultures
- Transient KRAS activation and induction of cell senescence
- Invertebrate genetic model lethality screens

V. Wrap-up and Summary
Moderator: C.J. Der, University of North Carolina, Chapel Hill

SESSION 2

I. C.J. Der, University of North Carolina, Chapel Hill: Welcome and day 1 review
II. Technical Approaches/Issues in RNAi
Moderator: B. Stillman, Cold Spring Harbor Laboratory, New York

- Targeting therapies
- Drug development and delivery
- RNAi library screening
- microRNA
- Gene arrays
- Proteomics
- Chemical libraries
- Phosphorylated proteins
- Secreted plasma proteins
- Genetic screens

III. N. Tonks, Cold Spring Harbor Laboratory: Wrap-up and summary
IV. Boxed Lunch—Continued Wrap-up
V. C.J. Der, University of North Carolina, Chapel Hill, **N. Tonks,** Cold Spring Harbor Laboratory, **R. Hruban,** John Hopkins Medical Institutions, Baltimore, Maryland: Summary/finalize research plan
VI. R.F. Vizza, The Lustgarten Foundation, Bethpage, New York: Closing remarks

D. Bar-Sagi

A. Saltiel, K. Shannon, J. Gibbs

Plant Genetics and Gene Regulation

September 7–10

FUNDED BY **Cold Spring Harbor–Pioneer Collaborative Research Program**

ARRANGED BY **R. Martienssen,** Cold Spring Harbor Laboratory
S. Tingey, DuPont Experimental Station

Welcome: **R. Martienssen,** Cold Spring Harbor Laboratory, and **S. Tingey,** DuPont Experimental Station, Wilmington, Delaware

Minisymposium on Plant Genetics and Development I

R.S. Poethig, University of Pennsylvania, Philadelphia: The regulation of leaf shape in *Arabidopsis thaliana*.
R. Simon, Institut fur Genetik, Düsseldorf, Germany: Regulation of plant stem cell fate by intercellular signaling.
J. Fletcher, USDA–University of California, Berkeley, Albany, California: Polycomb and trithorax activity in *Arabidopsis* organ formation: Role of the *ULT1* gene.
D. Jackson, Cold Spring Harbor Laboratory: Inflorescence architecture in maize.

S. Tingey, R. Martienssen

Minisymposium on Plant Genetics and Development II

X.-W. Deng, Yale University, New Haven, Connecticut: An initial analysis of maize epigenomes and their relationship with transcriptional activity.
P. Green, University of Delaware, Newark: Global analysis of miRNAs and miRNA targets.
B.C. Meyers, University of Delaware, Newark: Small RNAs of maize and beyond.
R. Williams, DuPont experimental Station, Wilmington, Delaware: Gene expression profiling of miRNAs.
R. Martienssen, Cold Spring Harbor Laboratory: Inheritance and reprogramming of heterochromatin with RNAi.

R.S. Poethig, M. Timmermans

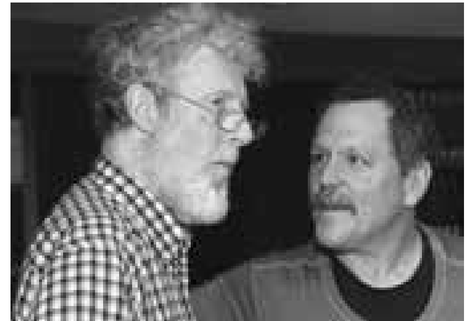
How Can We Improve Our Brains?

September 14–17

FUNDED BY **The Charles A. Dana Foundation**

ARRANGED BY
E.R. Kandel, Columbia University
W.T. Dickens, Russell Sage Foundation
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

It is the hope of every parent that their child will be bright and intelligent. Parents work to help their children's brain work better through providing education and stimulation, and society as a whole makes a tremendous commitment to the education of its young people. Are there data emerging from cognitive neuroscience that such education programs should take into account? Are there learning regimes that might be more effective than those typically found in the classroom? At the other end of life, is the hope of all of us that the normal decline in cognitive skills that accompanies aging will be slow? Might "brain exercises" maintain our brains at a higher level of functioning? Is there evidence that such exercises work? Are there effective pharmacological agents? In short, how can we best use the resources of society to help our brains work better throughout our lives?



J. Flynn, J. Schwartz

Welcome: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introductory Remarks: **J.D. Watson,** Cold Spring Harbor Laboratory

SESSION 1: Overviews
Chairperson: W.T. Dickens, Russell Sage Foundation, New York

E.R. Kandel, Columbia University, New York: We are what we remember: Memory and the biological basis of individuality.
D.K. Detterman, Case Western Reserve University, Cleveland, Ohio: General intelligence, achievement, and environmental effects.
R.E. Nisbett, University of Michigan, Ann Arbor: Intelligence and how to get it: Why schools and cultures count.

SESSION 2: Measurement
Chairperson: J.R. Flynn, University of Otago, Dunedin, New Zealand

C. Blair, New York University, New York: Improving fluid intelligence.
J.J. McArdle, University of Southern California, Los Angeles: Contemporary measurement issues in the evaluation of adult cognition.
W.T. Dickens, Russell Sage Foundation, New York: What is g?
R. Colom, Universidad Autonoma de Madrid, Spain: 4 + 2 ways to improve our brains.

D. Halpern E. Kandel

SESSION 3: Memory and Plasticity
Chairperson: E.R. Kandel, Columbia University, New York

M.C. Potter, Massachusetts Institute of Technology, Cambridge: Conceptual short-term memory and attention.
M. Merzenich, University of California, San Francisco: Brain plasticity-based therapeutics.
F.P. de Lange, Radboud University, Nijmegen, The Netherlands: Structural brain changes following psychotherapy.
J.M. Schwartz, University of California School of Medicine, Los Angeles: Nonreductionist approaches to neuroscience: Neuroplasticity for the coming immaterialist era.

SESSION 4: Differences
Chairperson: D.K. Detterman, Case Western Reserve University, Cleveland, Ohio

R.J. Haier, UCI Medical Center, Irvine, California: Neuroimaging studies of learning: Do all brains work the same way?
D.F. Halpern, Claremont McKenna College, California: Sex differences in intelligence and their implications for national/state educational policies.
T.A. Salthouse, University of Virginia, Charlottesville: Mental exercise and mental aging.
L.S. Gottfredson, University of Delaware, Newark: The fragility of maximal performance.
E. Turkheimer, University of Virginia, Charlottesville: The relationship between poverty and the heritability of intelligence.

L. Gottfredson, D. Detterman

SESSION 5: Early Education
Chairperson: C. Blair, New York University, New York

W. Steven Barnett, Rutgers, The State University of New Jersey: New Brunswick: Early education's effect on IQ and achievement.
C.T. Ramey, Georgetown University, Washington, D.C.: Preschool randomized controlled trials and population interventions to facilitate human competence.
L. Schweinhart, High/Scope Education Research Foundation, Ypsilanti, Michigan: The High/Scope Perry Preschool Study

through age 40.

M. Rosario Rueda, University of Granada, Spain: Enhancing brain function through cognitive training in young children.

J. Brooks-Gunn, National Center for Children and Families, New York: Long-term efficacy of early childhood education programs for poor children.

SESSION 6: Ability and Achievement
Chairperson: D.F. Halpern, Claremont McKenna College, Claremont, California

J.R. Flynn, University of Otago, Dunedin, New Zealand: Critical acumen: Step child of IQ tests and American education.

P.K. Kuhl, University of Washington, Seattle: How children learn: Can (should) we try to improve it?

D. Lubinski, Vanderbilt University, Nashville, Tennessee: Intellectually precocious youth with exceptional potential for scientific creativity: What we currently know about maximizing their development.

E. Hunt, University of Washington, Seattle: The workplace demands for cognition.

P.D. Zelazo, University of Minnesota, Minneapolis: Promoting the development of executive function prefrontal cortical function in children.

Where do we go from here?
Discussion Leaders:
E.R. Kandel, Columbia University, New York
W.T. Dickens, Russell Sage Foundation, New York

J. Watson, W. Dickens, M. Potter, R. Haier

Nutrient Sensing in Plants: What Can Other Model Organisms Tell Us?

September 21–24

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **A.M. Jones,** University of North Carolina, Chapel Hill
D.P. Schachtman, Donald Danforth Plant Science Center

Nutrient sensing in response to mineral or carbon deficiency and enrichment is an important area of biological research in multicellular eukaryotes. However, the sensing mechanisms and the components of the signal transduction pathways that connect sensing to response are poorly elucidated. Recent progress has been made using different model organisms, but this is still an emerging and somewhat fragmented field of research. Therefore, the aim of this meeting was to gather together experts in the plant field with experts using other model systems to identify parallels among eukaryotes that will help to advance nutrient-sensing research across organisms.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Nutrient Sensing/Sensors
Chairperson: W.B. Frommer, Carnegie Institute for Science, Stanford

J. Thevelein, Catholic University of Louvain, Belgium: Transceptor-mediated nutrient sensing in yeast.

M. Kielland-Brandt, Technical University of Denmark, Kgs Lynby: Model for transporter-like nutrient sensors: Sensing a chemical potential difference over a membrane.

W.B. Frommer, Carnegie Institute for Science, Stanford, California: Making sense of nutrient sensing with the help of FRET sensors.

F. Tamanoi, University of California, Los Angeles: The TSC/Rheb/TOR signaling pathway in fission yeast.

C. Meyer, Institut Jean-Pierre Bourgin (IJPB), Versailles, France: Role in nutrient signaling of the target of rapamycin (TOR) pathway in plants.

SESSION 2: Metal Sensing
Chairperson: M.L. Guerinot, Dartmouth College

S. Puig, University of Valencia, Spain: The yeast *Saccharomyces cerevisiae* as a model organism to study copper and iron deficiencies.

M.L. Guerinot, Dartmouth College, Hanover, New Hampshire: Metal homeostasis in *Arabidopsis*.

SESSION 3: Nitrogen Sensing
Chairperson: N. von Wiren, University of Hohenheim, Germany

G. Coruzzi, New York University, New York: A systems approach to nitrogen-regulatory networks and the virtual plant.
N. von Wiren, University of Hohenheim, Germany: Ammonium sensing in *Arabidopsis* roots.
B. Andre, Free University of Brussels, Belgium: Role of membrane transporters in amino acid signaling in yeast.
N. Crawford, University of California, San Diego: Signaling by inorganic nitrogen.
A. Gojon, Institut National de la Recherche Agronomique, Montpellier, France: Nitrogen sensing by NRT1.1 and its role in the regulation of root development in *Arabidopsis*.
B.G. Forde, Lancaster University, United Kingdom: Nitrate and glutamate sensing in *Arabidopsis* roots.
D.R. Bush, Colorado State University, Fort Collins: Anion regulation of root architecture.

SESSION 4: Sugar Sensing
Chairperson: A.M. Jones, University of North Carolina, Chapel Hill

M. Johnston, Washington University Medical School, St. Louis, Missouri: A glucose-sensing reticulum in *S. cerevisiae*.
J. Sheen, Massachusetts General Hospital, Boston: Glucose and energy signaling networks.
J. Rutter, University of Utah School of Medicine, Salt Lake City: Regulation of glucose partitioning by pas kinase.
A.M. Jones, University of North Carolina, Chapel Hill: Glucose sensing through a novel receptor GAP.
P. Leon, Instituto de Biotechnologia, UNAM, Cuervanaca, Morelos, Mexico: Role of AB14 during sugar signaling in *Arabidopsis* early seedling development.
S.C. Smeekens, University of Utrecht, The Netherlands: Sugar signaling and reprogramming of metabolism in plants.
C. Hoffman, Boston College, Chestnut Hill, Massachusetts: An Hsp90-Git7 requirement for glucose/cAMP signaling in *Schizosaccharomyces pombe*.

SESSION 5: Phosphate Sensing
Chairperson: S. Abel, University of California, Davis

J. Paz Ares, Centro National de Biotechnologia-CSIC, Madrid, Spain: Phosphate starvation signaling in *Arabidopsis*.
S. Abel, University of California, Davis: Phosphate sensing in root development.
T. Desnos, Centre National de la Recherche Scientifique, St. Paul-lez-Durance, France: Sensing low phosphate at the root tip.
L. Herrera-Estrella, Centro de Investigaciones y Estudios Avanzados, Irapuato, Guanajuato, Mexico: Phosphate availability alters lateral root development in *Arabidopsis* seedlings by modulating auxin sensitivity via a TIR1-dependent mechanism.
C. Xue, Duke University Medical Center, Durham, North Carolina: The human fungal pathogen *Cryptococcus neoformans* senses plant signals to complete its sexual cycle.
M. Bucher, University of Cologne, Germany: Signaling pathway cross-talk in mycorrhizal phosphate uptake.
T.-J. Chiou, Agricultural Biotechnology Research Center, Taipei, Taiwan, Republic of China: microRNAs in sensing phosphate availability.
W.-R. Scheible, Max-Planck Institute for Molecular Plant Physiology, Potsdam, Germany: Impact of small RNAs and long-distance signaling in the regulation of macronutrient responses.

SESSION 6: Potassium Sensing
Chairperson: D.P. Schachtman, Donald Danforth Plant Science Center, St. Louis, Missouri

A. Amtmann, University of Glasgow, Scotland: Linking K nutrition to metabolism and defense.
S. Luan, University of California, Berkeley: A calcium signaling pathway for low-potassium response in *Arabidopsis*.
D.P. Schachtman, Donald Danforth Plant Science Center, St. Louis, Missouri: Potassium signaling pathways in *Arabidopsis* roots.

SESSION 7: Conclusions and General Discussion
Chairpersons: A.M. Jones, University of North Carolina, Chapel Hill, and **D.P. Schachtman,** Donald Danforth Plant Science Center, St. Louis, Missouri

G. Coruzzi, D. Schachtman

Who Are We?: Kinship, Ancestry, and Social Identity

October 6–9

FUNDED BY **The Richard Lounsbery Foundation**

ARRANGED BY **A. Chakravarti,** Johns Hopkins University School of Medicine
M.W. Foster, University of Oklahoma
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Genetics provides, on the one hand, a description of human beings that reflects their *biological* ancestry. On the other hand, cultural norms provide a description (often by self-identification) of *social* ancestry. These two descriptions need not be, and often are not, the same. However, clinical geneticists identify populations that are likely to be genetically more homogeneous by grouping individuals according to their ethnic characteristics. And in recent years, there has been a proliferation of companies offering DNA-based genealogies, established by examining a set of DNA markers, but discrepancies among ancestries revealed by genetic analysis and assumed by cultural descent may profoundly affect individuals' views of themselves. The importance of the relationship between genetic and ethnic identities requires careful, rational, and critical review. The advent of ever cheaper high-throughput genomic techniques together with the proliferation of companies offering DNA-based ancestry analysis highlights the need to begin discussions of this topic.

M. Foster, J. Witkowski

Welcome: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Patterns of Global Human Variation
Chairperson: M.W. Foster, University of Oklahoma, Norman

L. Jorde, University of Utah, Salt Lake City: Genetics "race" and medicine.
S. Tishkoff, University of Pennsylvania, Philadelphia: Genetic variation in Africa.
J. Bertranpetit, Universitat Pompeu Fabra, Barcelona, Spain: The Basques in the European genetic landscape: Changing views from changing genetic data and with sociological factors.
H. Ostrer, New York University, New York: Who are the Jews? A 4000-year genetic perspective.

SESSION 2: Kinship, Relationship, and Ancestry: Definitions, Measurements, and Meaning
Chairperson: K.M. Weiss, Pennsylvania State University, University Park

M.A. Stoneking, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany: Genetic variation in worldwide human populations based on 1 million SNPs.
B.S. Weir, University of Washington, Seattle: F statistics and principal components for ancestry and kinship inference.
D.E. Reich, Harvard Medical School, Boston, Massachusetts: The genetic structure of 25 ethnolinguistically diverse Indian populations and their relationship to worldwide variation.

M. Olson, C. Bustamante, E. Thompson, L. Jorde, K. Weiss

SESSION 3: Who Is Related to Whom? Population Features
Chairperson: M.-C. King, University of Washington, Seattle

M.V. Olson, University of Washington, Seattle: The right way to view kinship is one genome segment at a time.
E. Thompson, University of Washington, Seattle: Inferring identity by descent from genomic SNP data in the absence of pedigree structure information.
J. Sinsheimer, University of California, Los Angeles: Determining ethnic admixture using the Mendel Software Package.
D. Serre, The Cleveland Clinic, Ohio: Clines, clades, and why it matters.
M.F. Seldin, University of California, Davis: Ascertaining and applying markers informative for population genetic structure and substructure for studies of complex traits.

SESSION 4: Who Is Related to Whom? Individual Features
Chairperson: R. Cook-Deegan, Duke University, Durham, North Carolina

I. Pe'er, Columbia University, New York: Whole genome, whole population mapping of hidden relatedness.
M.D. Shriver, Pennsylvania State University, University Park: Facial features, biogeographical ancestry, and admixture mapping.
D.B. Goldstein, Duke University, Durham, North Carolina: Rare and common variants in genetic history and genetic medicine.
C.D. Bustamante, Cornell University, Ithaca, New York: Global distribution of genomic diversity underscores rich complex history of continental human populations.
K.M. Weiss, Pennsylvania State University, University Park: How true is fiction?

SESSION 5: Ancestry, Race, and Complex Traits
Chairperson: M.W. Foster, University of Oklahoma, Norman

R.S. Cooper, Loyola University, Maywood, Illinois: Chronic disease has social causes.
C. Rotimi, National Human Genome Research Institute, Bethesda, Maryland: Health disparities: Is genomics a piece of the puzzle?

General Discussion
Introduction: M.C. King, University of Washington, Seattle
Moderator: M. Foster, University of Oklahoma, Norman

J. Bertranpetit, B. Weir

Oligonucleotide-directed Splicing: Therapeutic Strategies

October 14–17

FUNDED BY **Foundation to AVI, BioPharma, Inc., Cure Duchenne Foundation, Eradicate Duchenne, and Prosensa**

ARRANGED BY **E.P. Hoffman,** Children's National Medical Center
A. Krainer, Cold Spring Harbor Laboratory
T.A. Partridge, Children's National Medical Center

Although research and development of small sequence-specific oligonucleotides as small-molecule drugs have been pursued for 25 years, it has been rather disappointing. However, very recent studies have shown that oligonucleotides can be used to modify the splicing patterns of pre-mRNAs in genetic disorders to produce a functional mRNA. Such a strategy might be widely applicable. In recent years, there have also been advances in nucleotide chemistry that have led to oligonucleotides that retain sequence-specific antisense activity while showing little or no protein binding or associated off-target effects, whereas other developments have improved intracellular delivery in a larger variety of tissues and cells. In light of these recent advances, this meeting was held to critically review progress on oligonucleotides as therapeutic agents. The goal was to end the meeting with a clearer understanding of the hurdles that remain in using oligonucleotides as therapeutic agents and to highlight research strategies that are likely to be fruitful.

E. Hoffman, S. Takeda

Welcome: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introduction: **E.P. Hoffman,** Children's National Medical Center, Washington, D.C.

SESSION 1: Background
Chairperson: G.-J.B. van Ommen, Leiden University Medical Center, The Netherlands

G.-J.B. van Ommen, Leiden University Medical Center, The Netherlands: Cell animal and biomarker studies to improve exon skipping for DMD and extend the approach to other genes.
A. Krainer, Cold Spring Harbor Laboratory: Splicing correction as a therapeutic approach for spinal muscular atrophy.
R.T. Moxley, University of Rochester, New York: Myotonic dystrophy and exon skipping.
S.D. Wilton, University of Western Australia, Perth: Oligo design and evaluation: What can be expected?
L. Cartegni, Memorial Sloan-Kettering, New York: Modulation of alternative splicing in cancer.

P. Furlong, C. Miceli

SESSION 2: Preclinical
Chairperson: T.A. Partridge, Children's National Medical Center, Washington, D.C.

T.A. Partridge, Children's National Medical Center, Washington, D.C.: Exon choice, issues in detecting efficacy.
R. Kole, AVI BioPharma, Inc., Corvallis, Oregon: Splice switching oligomers: Technology progress and application to treatment of Duchenne muscular dystrophy.
S. Takeda, National Institute of Neuroscience, Tokyo, Japan: The significance of multiexon skipping of the dystrophin gene by morpholino treatment.
E.P. Hoffman, Children's National Medical Center, Washington, D.C.: Local and systemic delivery in dogs.
H. Chao, Mount Sinai School of Medicine, New York: Spliceosome-mediated RNA *trans*-splicing for gene repair: From hemophilia to muscular dystrophy.

SESSION 3: Clinical
Chairperson: F. Muntoni, UCL Institute of Child Health, London, United Kingdom

G. Platenburg, Prosensa, Leiden, The Netherlands: Prosensa Program.
F. Muntoni, UCL Institute of Child Health, London, United Kingdom: Current efforts on morpholino antisense studies in patients with Duchenne and deletions of exon 51.
P. O'Hanley, AVI BioPharma, Inc., Portland, Oregon: AVI's clinical strategy for developing PMO-based dystrophin exon-skipping drugs for DMD.
C.F. Bennett, Isis Pharmaceuticals, Inc., Carlsbad, California: Therapeutic opportunities for oligonucleotides that modulate splicing.

SESSION 4: Strategies to Increase Potency
Chairperson: F. Muntoni, UCL Institute of Child Health, London, United Kingdom

Q.L. Lu, Carolinas Medical Center, Charlotte, North Carolina: Is PPMO safe to use in clinical trial and what regime should be used?
S. Jiang, Gene Tools, LLC, Philmath, Oregon: Vivo-morpholino oligomers induce potent exon skipping of dystrophin in cardiac and skeletal muscles of mice.
T. Yokota, Children's National Medical Center, Washington D.C.: Exon skipping for dystrophic dogs.
S.F. Nelson, University of California Medical Center, Los Angeles, and M. Carrie Miceli, University of California, Los Angeles: HTS for enhancing exon skipping.
L. Garcia, Institute de Myologie, Paris, France: Dystrophin rescue by using exon skipping and/or *trans*-splicing approaches.

SESSION 5: Promoting the Pipeline in Novel Arenas
Chairperson: G.J. Vella, Charley's Fund, South Egremont, Massachusetts

B. Wentworth, Genzyme, Framingham, Massachusetts, and C. Nelson, Genzyme, Framingham, Massachusetts: Pathways to clinical trials.
J. Larkindale, Muscular Dystrophy Association, Tucson, Arizona: MDA.
B.D. Seckler, Charley's Fund, South Egremont, Massachusetts: Charley's Fund.
D. Miller, CureDuchenne, Corona del Mar, California: CureDuchenne.
W. Quirk, Foundation to Eradicate Duchenne, Inc., Alexandria, Virginia: FED.
P. Furlong, Parent Project Muscular Dystrophy, Middletown, Ohio: Parent Project.

Taking on New Complexities in SMA Biology

October 19–22

FUNDED BY **Spinal Muscular Atrophy Foundation**

ARRANGED BY
K. Chen, SMA Foundation
C.E. Henderson, Columbia University
T.M. Jessell, Columbia University
C. Joyce, SMA Foundation
M. Winberg, SMA Foundation

The goal of all those working on human genetic disorders is to develop therapies that will alleviate, if not cure, the disorder. This requires identifying therapeutic targets at different organizational levels and determining how best to reach those targets with current or new tools. To this end, the working sessions of this fourth SMA meeting were organized around three themes: Cellular targets (nerve muscle, glia, and their contacts); molecular targets (SMN2 and downstream molecules); and functional targets (SMA phenotypes in models and humans). The expectation was that the meeting would help to identify and promote collaborative experiments, as well as stimulate rapid translation of research ideas into therapeutics development and clinical research

K. Klinger, T. Jessell

Welcome and Introductions: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
D. Singh, SMA Foundation, New York and Organizing Committee
Introduction of Keynote Speaker: **D.C. DeVivo,** Columbia University Medical Center, New York
Keynote Talk and Discussion: **K.P. Campbell,** University of Iowa College of Medicine, Iowa City

SESSION 1: Cellular Targets
Chairpersons: M. Sendtner, Universitat Wuerzburg, Germany, and **G.J. Bassell,** Emory University, Atlanta, Georgia

G.D. Fischbach, Simons Foundation, New York: Reduced Ach release from type II SMA motor axons.
W. Thompson, University of Texas, Austin: Abnormal muscle development in a mouse model of SMA.
B.D. McCabe, Columbia University Medical Center, New York: SMN in *Drosophila*: Roles in NMJ Physiology.
J.M. Shefner, SUNY Upstate Medical University, Syracuse: Motor unit number estimation in a mouse model of SMA.
M. Sahin, Children's Hospital Boston, Massachusetts: RNA targets of SMN in axons.

SESSION 2: Cellular Targets
Chairpersons: W. Thompson, University of Texas, Austin, and **B.D. McCabe,** Columbia University Medical Center, New York

C.-P. Ko, University of Southern California, Los Angeles: Synapse loss in the SMN 7 mouse model of spinal muscular atrophy.
S.J. Burden, New York University Medical School, New York: Role of SMN1 in skeletal muscle.
G.Z. Mentis, NINDS/NIH, Bethesda, Maryland: Significant motor neuronal loss and altered synaptic input and excitability of lumbar motor neurons in SMA mice.
R.S. Finkel, Children's Hospital of Philadelphia, Pennsylvania: Electrophysiological evidence for impaired neuromuscular transmission in children with SMA.
E. Tizzano, Hospital Saint Pau, Barcelona, Spain: SMA during human development: Different pathogenic responses of muscle and motor neurons.

SESSION 3: Cellular Targets
Chairpersons: S.R. Jaffrey, Cornell University, New York, and **C.L. Lorson,** University of Missouri, Columbia

G.J. Bassell, Emory University, Atlanta, Georgia: Interactions of SMN with β-actin mRNA-binding proteins important to axonal growth.
C.E. Beattie, The Ohio State University, Columbus: Generating a genetic model of SMA in zebrafish.
M. Sendtner, Universitat Wurzburg, Germany: Valproic acid, a drug candidate for spinal muscular atrophy, blocks axon growth and excitability in motor neurons.
H. Wichterle, Columbia University, New York: Characterization of embryonic stem-cell-derived SMA motor neurons.
K. Eggan, Harvard University, Cambridge, Massachusetts: An in vitro model for SMA based on the differentiation of disease-specific human embryonic stem cell lines.

SESSION 4: Molecular Targets
Chairpersons: S. Paushkin, PTC Therapeutics, Inc., South Plainfield, New Jersey, and **K.H. Fischbeck,** NINDS/NIH, Bethesda, Maryland

L. Pellizzoni, Columbia University Medical Center, New York: SMN and pre-mRNA splicing.
C.L. Lorson, University of Missouri, Columbia: Readthrough-inducing compounds in SMA: SMN readthrough increases functionality compared to SMN_7.
S. Artavanis-Tsakonas, Harvard Medical School, Boston, Massachusetts: Modeling spinal muscular atrophy in invertebrates.
G.J. Lutz, Drexel University College of Medicine, Philadelphia, Pennsylvania: Preclinical studies of oligonucleotide-mediated SMN expression in mice with spinal muscular atrophy.
L.L. Rubin, Harvard University, Cambridge, Massachusetts: A high-content screen to identify molecules and pathways that regulate survival of motor neuron levels in motor neurons.

SESSION 5: Functional Targets
Chairpersons: K. Chen, SMA Foundation, New York, and **J.D. Porter,** NINDS/NIH, Bethesda, Maryland

C.J. Sumner, Johns Hopkins University, Baltimore, Maryland: Development of the motor unit in SMA mice and effects of HDAC inhibition.
P. Aebischer, Swiss Federal Institute of Technology, Lausanne, Switzerland: Gene therapy for SMA: Proof of principle in transgenic mice and scale-up issues.
K.W. Klinger, Genzyme Corporation, Framingham, Massachusetts: Possibilities for gene therapy in SMA.
P. Kaufmann, Columbia University, New York: The natural history of spinal muscular atrophy: Preliminary findings from the PNCR study.
M. Winberg, SMA Foundation, New York: Developments in mice: An update on Foundation preclinical activities.

Closing Remarks
Moderator: T.M. Jessell, Howard Hughes Medical Institute, Columbia University, New York

Epigenetics: Mechanisms and Regulation

December 7–10

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **S.L. Berger,** Wistar Institute
R. Shiekhattar, Center for Genomic Regulation
A. Shilatifard, Stowers Institute for Medical Research

The term "epigenetics" has been used very loosely to cover states ranging from dynamic, short-lived, chromatin-mediated regulation to long-term alteration of chromatin and other extrachromosomal proteins in nonreplicating cells. Given that epigenetics now encompasses such a diversity of phenomena, it was felt that a discussion meeting was needed to reassess what phenomena are epigenetic. Themes of the meeting included How shall epigenetics be defined—narrowly or broadly? What are the phenotypes associated with epigenetics? How are epigenetic states regulated? What are the links between epigenetics and human diseases? What are the understudied areas within epigenetics?

R. Martienssen, D. Moazed

Welcome: **S. Berger,** Wistar Institute, Philadelphia, Pennsylvania,
R. Shiekhattar, Center for Genomic Regulation, Barcelona, Spain
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri

SESSION 1: Definition of "Epigenetics" and Potential Mechanisms
Chairperson: R. Shiekhattar, Center for Genomic Regulation, Barcelona, Spain

Speakers
M. Grunstein, University of California, Los Angeles School of Medicine
S. Henikoff, Fred Hutchinson Cancer Research Center, Seattle, Washington

Discussion

Speakers
R. Kingston, MGH, Harvard Medical School, Boston, Massachusetts
M. Ptashne, Memorial Sloan-Kettering Cancer Center, New York
B. Stillman, Cold Spring Harbor Laboratory

Discussion

SESSION 2: DNA-binding Factors
Chairperson: K. Struhl, Harvard Medical School, Boston, Massachusetts

Speakers
G. Felsenfeld, NIDDK/NIH, Bethesda, Maryland
V. Pirrotta, Rutgers University, Piscataway, New Jersey
K. Struhl, Harvard Medical School, Boston, Massachusetts

G. Struhl

Discussion

Speakers
J. Widom, Northwestern University, Evanston, Illinois
R. Young, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
K. Zaret, Fox Chase Cancer Center, Philadelphia, Pennsylvania

Discussion

SESSION 3: DNA Methylation
Chairperson: M. Bartolomei, University of Pennsylvania, Philadelphia

Speakers
S. Baylin, Johns Hopkins University School of Medicine, Baltimore, Maryland
M. Bartolomei, University of Pennsylvania, Philadelphia
A. Bird, University of Edinburgh, United Kingdom

Discussion

Speakers
K. Helin, Copenhagen Biocenter, Denmark
R. Martienssen, Cold Spring Harbor Laboratory
P. Jones, University of Southern California, Los Angeles

Discussion

R. Kingston, M. Ptashne, A. Bird

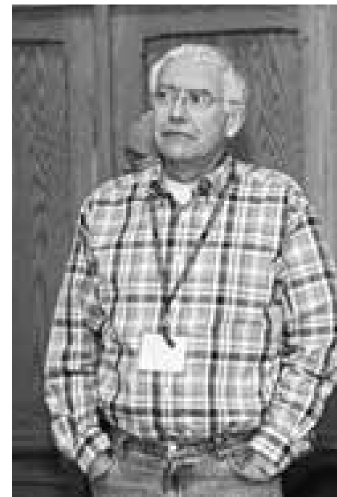
SESSION 4: Histone Modifications
Chairpersons: A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri, and **S. Berger,** Wistar Institute, Philadelphia, Pennsylvania

Speakers
S. Berger, Wistar Institute, Philadelphia, Pennsylvania
B. Bernstein, Broad Institute Pathology, Charlestown, Massachusetts
T. Kouzarides, University of Cambridge, United Kingdom

Discussion

Speakers
D. Reinberg, New York University School of Medicine, New York
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri
Y. Zhang, Howard Hughes Medical Institute, University of North Carolina, Chapel Hill

Discussion


P. Jones

SESSION 5: RNA AND RNAi
Chairperson: T. Kouzarides, University of Cambridge, United Kingdom

Speakers
S. Grewel, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
G. Hannon, Cold Spring Harbor Laboratory
M. Kuroda, Harvard University, Boston, Massachusetts

Discussion

Speakers
J. Lee, Massachusetts General Hospital, Boston
D. Moazed, Harvard Medical School, Boston, Massachusetts
R. Shiekhattar, Center for Regulation of Genome, Barcelona, Spain

Discussion

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
Lauren Correri	Elna Carrasco	Steven Blue	Cornel Ghiban
Mary Lamont	Jennifer Cutillo	John Connolly	Uwe Hilgert
Valerie Meszaros	Natalia Hanson	Eun-Sook Jeong	Bruce Nash
David Micklos	Malissa Hewitt	Susan Lauter	Jermel Watkins
Karen Orzel	Amanda McBrien	Chun-hua Yang	
Carolyn Reid	Erin McKechnie		
	Ileana Rios		
	Tedi Setton		
	Jason Williams		

The Dolan DNA Learning Center's 20th birthday, on September 18, inevitably sparked some reverie about our origins. It all actually began in 1984 with an innocent enough phone call from Fran Roberts, the new superintendent of Cold Spring Harbor School District, inquiring how we might join forces to do something to improve local science education. That conversation led to a proposal to the National Science Foundation (NSF) to develop a course to train high school teachers to do recombinant DNA experiments. Dejected when our proposal was turned down, I turned to Mike Glennon, principal, and Ed Tronolone, science chairman, of the Wheatley School in East Williston. They had a simple solution: Just ask several local school districts to fund the project!

I doubt that this tactic could have worked anywhere except on Long Island's North Shore, but by January 1985, eight local districts had contributed $10,000 apiece to purchase supplies and equipment. During the next several months, I spent spare moments in Rich Roberts' lab, working with postdoctoral fellow Greg Freyer to develop a simple set of experiments that would allow high school students to get their hands dirty with recombinant DNA. The experiments, which were the basis of our *DNA Science* lab text, were first tested in summer 1985 at a teacher-student workshop at the Wheatley School.

The first *DNA Science* Workshop spanned two weeks in summer 1985 and included nearly 40 participants.

Dave Micklos, Christine Bartels, and Jeff Mondschein before the Vector Van's maiden voyage across the U.S.

The Vector Van approaching the Golden Gate Bridge in San Francisco

In 1986, we obtained funding from Citibank to develop a mobile "Vector Van"—a Ford Econoline with a silver paint job and loaded to the ceiling with pipettes, centrifuges, and water baths. With independent funding from small foundations and biotech start-ups, we took our DNA show on the road, conducting week-long teacher workshops at six sites across the United States. On the last leg of the trip—from Milwaukee, Wisconsin, to Davis, California—the Vector Van was piloted by a German graduate student who had just received her driver's license, with a high school intern as copilot. That same year, we initiated a cooperative agreement with Carolina Biological Supply Company to develop reagents and kits to accompany the *DNA Science* curriculum.

It is worth mentioning that I was hired by Jim Watson to start the CSHL public affairs and development efforts, so I regarded the burgeoning education program as an advanced form of public relations. Jim supported my moonlighting because he knew it was time for the Laboratory to take the lead in fostering "DNA literacy" among the general public—just as it had led the way with professional scientists. This was a bold move at a time when high-level scientists, here and elsewhere, were expected to concentrate solely on their research.

By fall of 1986, four staff members—myself, Sue Lauter, Ellen Skaggs, and Mark Bloom—were crammed into two offices on the main floor of Grace Auditorium (now the Meetings and Courses office), from which we ran a frenetic fusion of public affairs, development, and educational outreach. Our modicum of success was not lost on Jim, who concluded that the time had quickly come for the education program to conform to his dictum of organizational evolution: "You get bigger, or you get smaller." He determined that we should take over an excessed elementary school on Main Street and convert it into a DNA museum. Of course, none of us really understood what this might entail, and I braced to answer tough questions when a proposal was brought to the Board of Trustees for discussion. But then Dave Botstein, outspoken chairman of biochemistry at Stanford University, rose to champion the proposal—and the scientific trustees fell in strongly behind him. However, the notion of a stodgy museum was roundly disdained, and all felt much better with the friendlier concept of a learning center.

With Fran Roberts paving the way with the Board of Education, we forged ahead with a lease/option to buy the neo-Georgian-style building from the Cold Spring Harbor School system. Beautiful on the outside, it had been subdivided into a warren of temporary offices. It charmingly retained all of the original features from its construction in 1925, including pint-sized toilets and a multipurpose auditorium with a proscenium stage and two basketball hoops. One small problem: We had no hard cash to pay the rent, let alone to convert this antique building into a habitat for high-level science.

With the help of CSHL Trustees, our luck turned in the spring of 1987. Mary Jeanne and Henry Harris called from a golf outing to say that they would make a major gift to help pay for the first 6 month's rent. Dave Luke worked behind the scenes to help us obtain core support from the Josiah Macy Foundation. We also achieved our first NSF grant, acquired a second Vector Van, and conducted summer workshops at 14 locations around the country.

In the fall of 1987, we began to renovate the building. Emblematic of our goal to be a "hands-on" science center, a 32-seat lab was the first space renovated. In the spring of 1988, we conducted the first lab field trips during which students cut and separated viral DNA or inserted an antibiotic resistance gene into bacteria. Soon, I was driving a 24-foot truck back and forth between Washington, D.C., loading and unloading pieces of an exhibit, "The Search for Life," which we had borrowed from the Smithsonian Institution's National Museum of American History. The exhibit's intricate system of aluminum trusses and theatrical lights occupied the entire front hall, gym/auditorium, and two of four original classrooms. For a time, I had to crawl on all fours under exhibitry to get to my office. Then, there were artifacts ranging from Audrey, the man-eating plant from the Little Shop of Horrors, to a recreation of the lab bench at which Stanley Cohen constructed the first recombinant DNA molecule. Against all odds, everything was in place for the official opening of the DNA Learning Center on September 18, 1988.

The rest, as they say, is history. Since that initial grant in 1987, we have maintained continuous NSF support, and we have been lucky to have had the help of numerous prestigious funders—including the National Institutes of Health (NIH), Howard Hughes Medical Institute (HHMI), William Randolph Hearst Foundation, Dana Foundation, William & Flora Hewlett Foundation, Burroughs-Wellcome Fund, Amgen Foundation, OSI Pharmaceuticals, and Pall Corporation. Major private donors—notably Helen and Charles Dolan, Claire and Ralph Landau, Laurie Landeau, Edward Chernoff, Doris and Peter Tilles, Sandra and Stephen Lessing, and Joan and Arthur Spiro—provided funds to reconstruct every corner of our fine old building and to assemble our first endowment. Together, these people and institutions have provided more than $30 million in support.

During the last 20 years, the DNALC has conducted experiments with a third of a million students—without fanfare or incident—proving the safety of DNA manipulation methods. Our *DNA Science* curriculum and kits provided by the Carolina Biological Supply Company brought modern DNA manipulation to millions more high school and college students. The DNALC's model for hands-on learning, Vector Vans, and local equipment-sharing have been replicated by numerous science centers, mobile vans, and "footlocker" programs in the United States and elsewhere. Thus, like its parent, Cold Spring Harbor Laboratory, the DNALC has projected a reputation that is considerably larger than its absolute size.

In retrospect, it seems improbable that we would have so successfully negotiated the risks of infancy and adolescence to emerge as a mature organization. Frankly, I doubt we could have reproduced this success elsewhere. Like others at CSHL, we clung tightly to the coattails of Jim Watson in the whirlwind of excitement he created for DNA, and we learned from Liz Watson that proper houses of science are rooted in our own history. Much of our success has flowed from the minds of Cold Spring Harbor Laboratory's scientists, who have shared with us their labs, their methods, and their infectious optimism. With their continued help, I suppose that our little center will survive another 20 years.

Biomedia Studio

A two-storey addition, completed in spring 2001, more than doubled the size of the DNALC. Included in the addition is the *Biomedia Center,* on the second floor, which has provided a purpose-built space to develop internet materials for biology education. Unfortunately, money ran out on the construction project, so for 7 years the western end of the addition remained an unfinished "attic" clogged with broken computers, excess lab equipment, exhibit parts, and even a life-size model of a New Guinea tribesman.

Bruce Stillman (*left*) and James Watson (right) look on as Laurie Landeau cuts the ribbon at the dedication of the Laurie J. Landeau Multimedia Studio.

A gift from CSHL trustee Laurie Landeau has allowed us to clear out the attic and finally complete this 1000-square-foot studio space as originally intended. On November 1, we celebrated our 20th anniversary with the opening of the *Laurie J. Landeau Multimedia Studio*.

The DNALC internet sites are already distinguished from many other science content sites by their extensive use of video, totaling nearly 20 hours of interviews with more than 200 scientists. Two new internet projects—*Genes to Cognition* (*G2C*) *Online* and the *iPlant* Collaborative—incorporate an equal volume. Equipped with theatrical lighting and high-definition digital cameras, the Landeau Studio will increase the professionalism of the DNALC's internet multimedia and allow us to embark on new productions. Two Sony XD-Cam high-definition video cameras record on a flash memory card and eliminate the time-consuming step of digitizing recordings made on digital video tape. A camera-mounted LiteRing and Chrommatte are the latest advancements of "greenscreen" technology, which allows us to edit a new background into the existing video recording. The studio provides three separately lit shooting locations: a desk for news productions, a coffee table and easy chairs for talk formats, and a lab table for experiment demonstrations.

With this infrastructure in place, we can now work with other CSHL departments to take fullest advantage of our unparalleled scientific resource—the more than 8000 scientists who visit each year to attend meetings and courses. These thought leaders in basic and applied genetics research can be efficiently shuttled for interviews from the main campus or Banbury Center. In addition to adding high-quality video to DNALC and other CSHL internet sites, the studio will provide video-conferencing capability that can keep us in touch with the thousands of teachers who use DNALC methods. It also offers the potential to expand our *DNA Today* concept to provide regular podcasts, vodcasts, and news feeds for the cable industry.

The Landeau Studio also includes a video/sound editing room and a conference/green room. This reconfiguration allowed us to redevelop the former conference area as a "living room" for DNALC staff. This stylish space, with a central free-form concrete lunch counter, was conceived by DNALC multimedia designer Eun Sook Jeong, who also has a master's degree in interior design. Together with the existing design bays and staff offices, the "living room" and Landeau Studio present a unified vision of a multimedia production facility for the 21st century.

iPlant Collaborative

During the past several years, the DNALC has worked with CSHL plant scientists to provide outreach components for a number of research projects funded by the NSF. Several of these projects came under the National Plant Genome Initiative to determine the entire sequence of corn and rice chromosomes. In

February, this work culminated in the receipt of a $2.1 million subaward of a $50 million consortium grant with the University of Arizona. The project will develop a national computer infrastructure to support plant research. In an allusion to Apple's success in making computing more personal, the *iPlant* Collaborative aims to develop tools and interfaces that will place large-scale plant data sets and high-powered informatics tools on any scientist's computer desktop.

After a spring kickoff symposium at Cold Spring Harbor Laboratory, the *iPlant* Collaborative hosted a series of six workshops for plant and computation researchers to brainstorm "grand challenges" in plant science. These workshops were hosted at Biosphere II, in Arizona, and drew together a multidisciplinary group of computational scientists and researchers working at all levels of plant function and organization: molecular biology, genetics, genomics, biochemistry, cell biology, physiology, plant breeding, systematics, ecology, and evolutionary biology. Beginning in 2009, *Grand Challenge* Teams will work with computer scientists at the University of Arizona and CSHL to develop a cyberinfrastructure of *Discovery Environments*, where plant researchers can share and analyze data in an unprecedented fashion. As a member of the *iPlant* Education, Outreach, and Training (EOT) component, the DNALC will work with each project team to create educational interfaces to the scientific tools and databases. In this way, students and teachers can work with the same data, using the same tools, and at the same time as high-level plant researchers. This will enable students to literally look over the shoulders of plant researchers as they, for example, understand the mechanisms that could enable crop plants to rapidly respond to global climate changes.

DNALC multimedia staff embarked on an ambitious project to help publicize the *iPlant* project and to document the *Grand Challenge* development process. We set up a mini-video studio at the *iPlant* meeting at Cold Spring Harbor Laboratory, at each of six *Grand Challenge* workshops at Biosphere II, and at the American Society of Plant Biology Meeting in Merida, Mexico, conducting a total of 143 interviews with plant and computational biologists. From these, we extracted 538 video clips that present a range of viewpoints on challenges in plant biology, cyberinfrastructure, and education. A video header on the *iPlant* internet site (http:// www.iplantcollaborative.org/) randomly loads clips or allows one to select specific interviews.

At year's end, we received approval from *iPlant* management to embark on an EOT demonstration project to develop an educational platform for gene annotation and comparison. Annotation is the process of discovering the biological information contained in a DNA sequence, viewing genes in the context of a chromosome, and relating them to other organisms. The analysis of DNA sequence entails a number of cumulative steps—assembling short sequences into "contigs," predicting genes and other functional elements, and merging gene evidence from RNA and other species—which is termed a "pipeline" or "workflow." Although masses of free DNA and gene data are available, each step of the workflow requires a different tool, and various tools and data sources often are not compatible. Our objective is to develop an easy-to-use interface that will allow students to easily find DNA data and seamlessly move it through several key stages of the annotation pipeline. We envision a desktop object that merges attributes of our

Biosphere II in Arizona made a stunning backdrop to the *iPlant* Collaborative *Grand Challenge* workshops.

The video interface at the *iPlant* Collaborative website includes interview clips of 143 plant and computer scientists.

BioServers (http://www.bioservers.org/bioserver/) and Geneboy (http://www.dnai.org/geneboy/) applications. Sarah Elgin (Washington University in St. Louis), Sue Wessler (University of Georgia), and Steve Slater (University of Wisconsin) are key collaborators on this project.

Genes to Cognition (G2C) Online

During the year, we pushed to complete *Genes to Cognition* (*G2C*) *Online*, our internet site on modern neuroscience research (http://www.g2conline.org). This site allows students to explore how thinking and disorders of thinking result from interactions at various levels of biological function—from genes, to biomolecules, cells, physiology, behavior, and environment. We completed 40 video interviews and demonstrations with neuroscience researchers from the United States and Europe, creating more than 300 new video clips and animations for launch in March 2009. A major goal of *G2C Online* is to increase understanding of the process of science. To this end, we collaborated with researchers at the National Institute of Mental Health to develop three video modules on neuroimaging based on fMRI, MEG, and PET technologies. In these demonstrations, researchers introduce the techniques, guide users through an experiment, and discuss data.

We continued to develop touchstone animations that highlight key concepts of brain science. Following up a recommendation from our Advisory Panel, we collaborated with AXS-3D, a Toronto-based animation studio, to develop the *G2C Brain*. This unique interface allows one to rotate a three-dimensional

Genes to Cognition Online is scheduled to launch during the Dana Foundation Brain Awareness Week in March 2009.

The 3-D brain at *G2C Online* includes information on 29 structures with associated disorders and links to current research. In *Fly School*, visitors can perform an experiment online to test learning in *Drosophila*.

model to explore the structure and function of 29 parts of the human brain. Each structure or region includes information on associated disorders, brain damage, case studies, and links to contemporary research. *Molecules for Memory* was developed to supplement a video interview with Nobel Laureate Eric Kandel. In this animation, users can trace the formation of a long-term memory from the generation of presynaptic action potentials, through protein–protein interactions in the postsynaptic neuron, to the formation of new synapses. This animation joins a series of high-value pieces, including the *Neural Code*, a proteomics demonstration; *Memory Lanes*, a demonstration of how cab drivers remember routes; *Gene Knockout*, an explanation of how to alter genes in mice; and the *Fly School* and *Swimming Mice* interactive experiments. In addition to animations and video clips, we have harvested an extensive library of articles from the Dana Foundation and a 300-word glossary.

We also collaborated with Seth Grant's group at the Wellcome Trust Sanger Institute to develop a protein–protein interaction database (PPID) browser. This is a map tool that allows one to explore a network of 105 proteins involved in synaptic transmission. Clicking on any one of the proteins calls up a customized abstract with links to *G2C Online* content and detailed information (the Allen brain atlas, NCBI gene information, and *Ensembl* homology links). The browser bridges the gap between science and education, allowing researchers and students to toggle between technical and popular information about a particular protein.

G2C Online's unique site architecture extends networking software developed at MIT's *Media Lab*, which displays content as an interlinked network of dimensional content "atoms." One can browse the network by zooming in and out, following links between adjacent atoms, and clicking on any atom to call up multimedia items. Our three-dimensional network is based on two-dimensional concept maps that were developed from interviews with leading neuroscience researchers. In 2008, we redeveloped our browser software as *Simple Mapper*, which allows users to build their own network maps and then link atoms to online content from *G2C* or other sources. In addition to creating striking network maps, *Simple Mapper* can also be used to generate concept maps to assess student learning.

In the spring, Amy Niselle, a doctoral student from the University of Melbourne, joined the *G2C Online* project to assist with program evaluation. She worked extensively with Danielle Sixsmith to conduct a

detailed usability study with 48 Advanced Placement (AP) biology and psychology students. Data were collected on how students interacted with the internet site, how they accessed relevant content, and how the site helped or hindered learning. Insights from this study led to several important changes in site design and navigation. Notably, we enabled our Flash interface to be controlled by browser back/forward buttons (not a trivial project), and we streamlined text for easy browsing and skim reading.

Inside Cancer

With Phase I funding from an NIH Science Education Partnership Award (SEPA), we developed *Inside Cancer* (www.insidecancer.org), a multimedia internet resource for understanding the molecular genetic basis of cancer. By focusing on how researchers gain insights into the unseen world of genes and signaling molecules, *Inside Cancer* provides examples of the science process. With Phase II funding, we are implementing a number of improvements that support and extend syllabus-centered teaching. Notably, we disaggregated *Inside Cancer* content by breaking it into individual content “atoms.” Each atom was then entered into a content management system and annotated with metatags, including alignment with national and state educational standards. Using this system, a teacher can identify sets of atoms that illustrate particular content standards or, conversely, collect items that respond to various standards as they browse the site.

We also developed a companion *Teacher Center* that includes standards alignments, as well as a tool that allows teachers to create custom presentations with *Inside Cancer* content, and a wiki for developing and exchanging lesson plans. Faculty Fellows Naomi Cook (Horace Greeley High School), Sue Holt (Life Sciences Learning Center, University of Rochester), and Glen Cochrane (lead biology teacher, Half Hollow Hills High School) developed sample lessons to “seed” the wiki. Naomi spent an extended period at the DNALC adapting multimedia elements from other DNALC sites to expand content at *Inside Cancer*.

Maize Internet Sites

During the year, we collaborated with CSHL researchers Dick McCombie and Doreen Ware, and Rick Myers of Washington University in St. Louis to develop a mini-website describing the NSF-funded Maize Genome Sequencing Project (http://www.dnalc.org/maize/maize.html). The site features interviews with scientists involved in deciphering the information in the maize genome, a tour of the genome sequencing center at Washington University, an animation of the sequencing and assembly process, and a video tutorial for using the online maize sequence browser.

In the fall, our work in plant education was further bolstered with receipt of an outreach grant from the maize meristem collaboration between CSHL faculty member Marja Timmermans and scientists at Cornell University. Under this project, the DNALC will develop a multimedia internet site, *Weed to Wonder*. We will tell the remarkable story of the corn plant’s amazing rise—from a common weed, to staple food and religious icon of Native Americans, to modern hybrid cultivar, to versatile and ubiquitous component of processed food, to precursor of clothing and motor fuels, to pharmaceutical factory. *Weed to Wonder* will celebrate this uniquely American success story and provide a case study of the interaction among science, technology, and society (STS). The site will open an internet window on modern research on plant genomes, as well as a time machine back into the social and scientific history of agricultural breeding. The goal will be to show the continuity of research on corn—from Native American agriculturalists to agricultural breeders, corn geneticists, plant physiologists, and molecular biologists—that has culminated in the Maize Genome Sequencing Project. In parallel with the story of human impact on the evolution of the corn plant, we will also tell the story of corn’s impact on human culture.

Weed to Wonder will capitalize on the internet’s capacity to juxtapose different media, providing options to engage people with different interests and learning styles (“intelligences”). The major site will be complemented by serialized podcasts in audio and video formats. Video interviews will provide contrasting vignettes of people involved with corn at various levels. Researchers will provide understanding of the process of science, and interviews with farmers, historians, and ethnographers will portray corn’s role in society. Animations will be especially effective in describing experiments and bringing to life unseen

The Weed to Wonder site will explore how humans change corn and how corn changed human society.

molecular events. Multimedia content will be integrated with online experiments and bioinformatics tools primarily developed by the DNALC with previous NSF funding. A continuum of practical experiments will allow middle school, high school, and beginning college students to progress from classical genetic analysis of kernel traits, through molecular genetic and bioinformatic analysis of mutations, to the examination of genome structure and evolution.

Internet Visitation

Visits to the DNALC's family of internet sites decreased to 6.06 million in 2008—84% of the previous year's traffic. Although there were fewer visitors, those who came spent more time and accessed more content than in the previous year. Ten of 12 sites saw increases in the average viewing time and served nearly 4000 gigabytes (4 terabytes) of data, a 23% increase over 2007. *DNA Interactive*, our richest multimedia site, alone served a total of 1.3 terabytes.

As part of a concerted effort to improve visitation, we employed LunaMetrics, a company that specializes in search engine optimization. After analyzing a representative internet site (*Inside Cancer*), the consultants suggested ways to improve our metadata descriptions to make it easier for search engines to index content. Because search engines give preferred rankings to sites with frequent updating, the consultants also suggested adding blogs or news feeds to provide fresh content. We have been using these and other insights to renovate older sites and to guide development of new sites. Many of our popular sites have a linear story line, which makes it difficult for users to find particular multimedia atoms (animations and videos) to answer a specific question or instructional need. We are in the midst of a major project to disaggregate linear content and provide improved metadata so that individual videos and animations can be retrieved via a search, such as Google or Yahoo. Our new *G2C Online* site was specifically developed as a nonlinear network of atoms with associated metadata.

We continue to get a boost from a grant from Google AdWords, a free advertising program that displays sponsored links on Google's search results page. Our AdWords account contains a set of keywords for each DNALC website. A search for one of the keywords displays an ad for a DNALC site as a user "impression," and a "click-through" is logged when a link is followed. *DNA from the Beginning* is our most successful site on AdWords, logging 5,587,424 impressions and 103,294 click-throughs. In total, DNALC sites registered 13,441,908 impressions and 175,161 click-throughs, valued at $113,224.

	Average visit length	Change from 2007	Average monthly bandwidth (gigabytes)	Visits in 2008	Change from 2007 (percent)
Content-based sites					
Gene Almanac	9:13	+0:40	94.77	1,629,334	–15.28
DNA from the Beginning	9:32	+1:00	37.09	1,302,136	–10.29
Your Genes, Your Health	8:12	+0:32	41.45	805,683	–16.26
DNA Interactive & myDNAi	10:26	+2:02	111.56	1,151,103	–21.15
Image Archive on the American Eugenics Movement	17:46	+1:18	11.55	607,973	–16.93
Inside Cancer	7:18	+0:09	31.35	239,942	+22.33
Inside Cancer Teacher Center	14:45	n.a.	3.03	22,251	n.a.
Laboratory/bioinformatics sites					
BioServers	24:07	+7:57	2.56	135,955	–45.97
Genetic Origins	7:28	+0:24	1.57	102,738	–22.61
Greenomes	3:15	–0:12	0.54	13,312	+40.97
Dynamic Gene	7:18	+0:58	0.34	11,277	+41.21
Silencing Genomes	12:45	+4:34	0.55	29,461	+142.12
DNALC Kits/Carolina Collaboration	15:18	–0:08	2.34	5,425	+23.41
All sites	**9:27**			**6,059,590**	**–15.17%**

Faculty Training

During the year, more than 800 educators participated in professional development activities conducted at sites around the United States and Europe. With funding from the NSF, NIH, and the Hewlett Foundation, we collaborated with 14 host institutions to conduct 17 one- to five-day workshops on plant genomics, bioinformatics, cancer biology, RNA interference, and neurobiology. Five of these workshops were conducted at institutions with high proportions of underrepresented minorities, where 19% of participants were African American or Hispanic. Through our HHMI-sponsored collaboration with the New York City Department of Education, 105 teachers participated in workshops at the *Harlem DNA Lab*. An additional 428 educators attended workshops at professional meetings in the United States and Europe that covered topics including neurobiology, bioinformatics, the molecular genetics of taste and smell, detecting genetically modified foods by PCR, DNALC online tools for education, and DNA forensics.

At the end of July, 18 teachers from ten states attended the *Amgen Leadership Symposium in Human and Genomic Biology*. The first 2 weeks of the symposium included experiments and bioinformatics exercises on RNA interference (RNAi) and genetic modification of plants. Participants explored human genetics by genotyping polymorphic sites in their own DNA: (1) An *Alu* "jumping gene" insertion on chromosome 16 and single-nucleotide polymorphisms (SNPs) in the mitochondrial chromosome were used to study theories of human origins and ancient migration. (2) SNPs in a taste receptor were correlated with the ability to taste the bitter chemical phenylthiocarbamide (PTC). (3) Variations in a repeat region and analysis on a DNA chip illustrated state-of-the-art methods in forensic identification. In the final week, participants worked independently or in groups to develop lesson plans, optimize experimental methods for classroom instruction, screen supermarket foods for genetic modifications, expand bioinformatics exercises, and observe DNALC methods for instructing middle school students.

We continued to provide leadership training under our long-term collaboration with the Singapore Ministry of Education. Initiated in 2000 with a visit by Minister Teo Chee Hean, the collaboration established licensed DNA learning centers at the Singapore Science Center and National Institute of Education. Two junior college teachers and one high school teacher participated in the summer *Leadership Symposium*, and three elementary school teachers came in November for a 2-week stay. Besides hands-

Leadership Symposium educators and DNALC staff pose for the traditional class photo.

on lab work and preclass preparation, the November contingent observed and co-taught labs alongside DNALC instructors and visited a local elementary school. Each teacher developed a plan for translating their experience into hands-on instruction for Singaporean students.

Concluding Two Plant Outreach Projects

We concluded our collaboration with CSHL researcher Marja Timmermans and Cornell University's Mike Scanlon to encourage underrepresented minorities to teach and study plant genomics. In past summers, two Faculty Fellows spent 2 weeks working in plant genome labs at CSHL or Cornell and 1 week working with us at the DNALC. In 2008, we conducted 1.5-day follow-up workshops hosted by Faculty Fellows Gokhan Hacisalihoglu and Diomede Buzingo at their home institutions—Florida Agricultural & Mechanical University (Tallahassee) and Langston University (Oklahoma). Of the 25 high school and college educators who participated in these workshops, 24% were African American or Hispanic, mirroring their proportion among American residents (2005 census figures). Building on previous 3-day workshops at these sites, the follow-ups introduced educators to mobile "footlockers" that include PCR and electrophoresis equipment. Experiments use this equipment to test for genetically modified foods or analyze transposon polymorphisms in maize and *Arabidopsis*. Participants can borrow the equipment from host institutions for use in their classes, removing a major barrier to the implementation of modern experiments in plant molecular genetics. This project was summarized in an article written by Dr. Hacisalihoglu entitled *An Innovative Plant Genomics and Gene Annotation Program for High School, Community College, and University Faculty* (*CBE Life Sci. Educ.* **7:** 310–316).

We also completed work with Lincoln Stein and Doreen Ware on their NSF-funded *Gramene* project, which provides informatics tools for analyzing and comparing grain genomes. During the project, we developed the internet site *Dynamic Gene* (www.dynamicgene.org) and trained 304 high school and college faculty to use the site to annotate rice genes in their classes. Animated tutorials in the site explain how DNA sequences encode information, how computers identify patterns that predict gene structures, and how experimental evidence complements computer predictions to correctly identify genes. The *Annotation* section provides detailed instructions on how to analyze a predicted gene with *Apollo*, research software developed to analyze the *Drosophila* genome.

The *Projects* section allows students to download sections of rice chromosomes, annotate predicted genes, and upload their results to compare with classmates or share with researchers. This provides powerful opportunities for students to contribute to science by analyzing up-to-date biological data. This became evident during the fall of 2008, when we used the site with Cold Spring Harbor High School students enrolled in *Genomic Biology*, an advanced course offered at the DNALC. After 5 hours of instruc-

tion using *Apollo* and other online bioinformatics tools, student groups were charged with the task of identifying and evaluating genes in different regions of rice chromosome 1. By analyzing the available evidence, the 17 students were able to improve on the majority of computer-generated gene predictions and many previously annotated genes in a 400,000 base pair region. Remarkably, students were also able to identify four putative genes that had not previously been identified. Thus, we feel that certain novice high school and college students can contribute to our knowledge of gene structure, while gaining first-hand experience in modern bioinformatics.

Three New Teacher Training Workshops

RNA interference (RNAi) provides a powerful tool to move directly from DNA sequence to the analysis of gene function in living organisms. With Phase II funding from NSF's Course, Curriculum, and Laboratory Improvement (CCLI) program, we expanded our efforts to bring compelling RNAi experiments into college classrooms. Five-day *Silencing Genomes* Workshops were conducted in summer 2008 at Rust College (Holly Springs, Mississippi), Austin Community College (Texas), and the Fralin Biotechnology Center at Virginia Tech (Blacksburg, Virginia). Sixty-three college instructors were trained to perform RNAi in the model eukaryotic organism *Caenorhabditis elegans*. After observing mutant phenotypes and learning basic worm "husbandry," faculty used simple methods to induce RNAi and "single-worm PCR" to examine the mechanism of RNAi—comparing the DNA of worms with identical phenotypes induced by either RNAi or a mutation. They also learned open-ended methods that support student projects, using bioinformatics to identify a target gene and then to develop their own RNAi reagents "from scratch."

Using these methods, workshop participants collaborated to develop new targeting vectors designed to silence 40 *C. elegans* genes; these will be supplemented by another 60 vectors to be developed in workshops planned for the summer of 2009. Each vector has been entered into the *Silencing Genomes Projects* page and includes its own evolving record of how it was developed and how it is used by other students and teachers. A collection of bacterial and *C. elegans* strains, including these newly developed reagents, are freely available through our online strain order system. In 2008, 88 strains were sent to teachers in the United States, Thailand, Brazil, Poland, Singapore, and Spain to support teaching the *Silencing Genomes* curriculum, reaching an estimated 2600 students.

Ninety-two high school and college faculty learned to use internet content on cancer cell biology in health, general biology, and AP biology classes at six *Inside Cancer* workshops. One-day workshops supported through the NIH SEPA program were held at Stowers Institute for Medical Research (Kansas City, Missouri), Midland College (Texas), Fred Hutchinson Cancer Research Center (Seattle, Washington), and Kimmel Cancer Center (Philadelphia, Pennsylvania). Two workshops were also conducted in conjunction with teacher professional conferences: National Association of Biology Teachers (Memphis, Tennessee) and Biolink Southwest Region (Albuquerque, New Mexico).

With the support of the Hewlett Foundation, we instituted a nationwide workshop program to disseminate *G2C Online*. Workshops were held at the DNALC, the Stowers Institute (Kansas City, Missouri), Fred Hutchinson Center (Seattle, Washington), and St. Louis Science Center (Missouri); 61 high school and college educators participated in 1.5-day workshops introducing up-to-date neuroscience research, our library of multimedia items, and interactive experiments. Of these participants, 97% rated workshop content as excellent or good, and mean scores on pre- and post-knowledge tests rose from 6.57 to 8.91.

John Connolly (*standing*) leads a *G2C Online* educator workshop.

Harlem DNA Lab

In 2008, we fulfilled our long-held goal of developing a base of operations to provide underserved schools in New York City (NYC) enrichment opportunities identical to those offered to Long Island's affluent schools. In 2007, NYC Schools Chancellor Joel Klein provided us exclusive use of a 1200-square-foot

Chancellor Joel Klein was a featured speaker at the *Harlem DNA Lab* opening in September.

classroom in the John S. Roberts Educational Complex (JHS 45) in East Harlem. Then, in winter 2007–2008, the Division of School Facilities renovated the space according to our specifications, including our signature student lab desks. With a $100,000 grant from the Jerome L. Greene Foundation, we purchased equipment for elementary through advanced experiments—from dissecting and compound microscopes to a fast PCR machine and three Agilent Bioanalyzers that allow us to type student polymorphisms in a single 3-hour lab. One area of the lab is specifically designed for assembling footlockers and restocking reagent kits to be used in NYC schools.

Lab field trips began in the spring, and *Harlem DNA Lab* was dedicated on September 23 by NYC Schools Chancellor Joel Klein, Howard Hughes Medical Institute (HHMI) Vice President Peter Bruns, and CSHL President Bruce Stillman. The event was covered in the *New York Post*, and a video piece on NY1 featured students from the Emily Carey School.

Located at John S. Roberts Middle School, at First Avenue and 120th Street, *Harlem DNA Lab* offers the same range of high school lab field trips as offered at our Cold Spring Harbor and Lake Success facilities: bacterial transformation, DNA restriction analysis, forensic DNA profiling, and human mitochondrial DNA sequencing. Just over 1400 students participated in lab field trips in 2008. We extended our

Bruce Stillman and Dave Micklos talk with students at the *Harlem DNA Lab* opening.

CSHL Trustee Mary Lindsay watches as students spool DNA in a test tube.

popular *Genetics as a Model for Whole Learning* (*GMWL*) to every John S. Roberts student, providing three labs for each of 375 students in grades 6–8. A grant from the William T. Porter Foundation provided scholarships for John S. Roberts students, as well as 812 students participating in field trips and summer camps.

The capstone event for *Harlem DNA Lab* was the 2-week New York State Excelsior Scholars Program, which involved 39 8th graders from Manhattan, Queens, and the Bronx. Excelsior Scholars were selected based on standardized test scores that placed them in the top 10% of their science/math classes, teacher recommendations, and personal statements. A week-long *DNA Science* camp provided intensive lab experience for 16 high school students from around New York City.

HHMI Teacher Professional Development

Harlem DNA Lab is also the site for a city-wide professional development program funded by HHMI. The goal is to develop a base of teachers who can competently introduce science students to six “targeted” experiments highlighting key techniques in genetics and biotechnology. The experiments embody key concepts and process skills of the New York City Scope and Sequence for Science, New York State Science Core Curriculum, AP Biology, and *National Science Education Standards.*

During the first year of the project, we developed a strong working group composed of DNALC and Department of Education staff, key advisors, and six Faculty Fellows: Greg Borman, lecturer in middle/secondary science education, City University of New York and former science coordinator, Henry Street Middle School, Manhattan; Caren Gough, New York State Science Mentor, consultant, and adjunct faculty, Stony Brook University; Dr. Dahlia McGregor, biology teacher, South Shore High School, Brooklyn; Adrienne Rubin, biology teacher, Eleanor Roosevelt School, Manhattan; Kathleen Rucker, biology teacher, Brooklyn International High School; Jerry Watkins, retired science teacher, Central Islip Union Free School District (UFSD), Long Island.

During the week of August 4–8, the Fellows convened at the DNALC to help develop *Lab Center*, an internet site that specifically supports instruction of the six “targeted labs.” *Lab Center* represents a virtual classroom into which a DNALC staff member enters to make a video introduction to each lab. By clicking on objects on the whiteboard and desk, students can access (1) interactive and PDF versions of each experiment, (2) follow-up activities, (3) scientist interviews, (4) animations, and (5) selected links. Fellows developed formatted lesson plans that include prelab student work, detailed teacher tips, a timeline to complete the lab in 40-minute periods, and creative postlab activities. Their input helps to ensure that each lab builds logical connections between concepts and anticipates use in a variety of classroom settings.

The HHMI project has the aggressive goal of providing 24 hours of certificate training to 200 teachers per year. Thus, in 2008, we devoted considerable effort to developing relationships with key individuals and networks that can help us to recruit teachers. Through our collaboration with the Department of Education, our training schedule and registration materials are posted on major NYC registries for teacher development activities.

Visitation and Student Instruction

Annual visitation reached a new high of 40,613 in 2008. This included 20,043 students who conducted experiments at the DNALC, DNALC *West*, or the *Harlem DNA Lab*, and 8235 students who received in-school instruction by DNALC staff members. More than half of all experiments were conducted by middle school students under the banner of *GMWL*, which continues to offer quality field trip and in-school programs for students in grades 4–8. In 2008, more than 70 public and private schools were involved in the *GMWL* program.

Summer camps conducted at the DNALC, DNALC *West*, *Harlem DNA Lab*, Central Islip High School, and SUNY Old Westbury drew 921 students. We continued our collaboration with *Project Grad Long Island*, a nonprofit organization that works within economically disadvantaged students to increase high school graduation rates. *Project Grad* provided daily bussing for 22 high school students from the West-

bury UFSD to attend a week-long camp at the SUNY Old Westbury campus. This course, which included hands-on experiments on advanced topics in DNA science and human and plant genomics, was supported by a grant from the Keyspan Foundation. Arrow Electronics provided scholarships for four 5th grade students from the Westbury UFSD to attend *Fun with DNA* camps at the DNALC. As part of a continuing collaboration with Central Islip School District, Bank of America Long Island funded a *Fun with DNA* camp for 18 students from the Charles Mulligan Elementary School.

In 2008, we continued the *Great Moments in DNA Science* tradition with 425 high school student participants. Dr. Lloyd Trotman talked about the impact of varying *PTEN* expression levels in mouse models for prostate cancer. Dr. Bill Tansey explained how proteolysis contributes to important events in the cell and how abnormal proteolysis contributes to cancer. Dr. Doreen Ware described her work in understanding plant genome organization, evolution, and diversity. *Saturday DNA!* drew 272 student and adult participants for workshops such as *Survival of the Fittest*, *Cancer: Inside Out,* and *How to Solve a Crime*.

Our collaboration with the Watson School of Biological Sciences continued in 2008 with a large class of 14 graduate students. This rotation provided the graduate students an opportunity to learn skills for communicating with nonbiologists. During 12 half-day teaching sessions, pairs of students worked with expert DNALC instructors to develop presentation methods that can help convey unfamiliar ideas to people of any age or background. During the first phase of training, each student pair "shadowed" a DNALC instructor as he/she taught a middle or high school lab and then developed a lesson plan that integrated their perspectives into the formatted lab. In the second phase, each pair worked closely with DNALC instructors to co-teach several labs, developing their communication skills and preparing them for independent instruction. During the final phase, each pair was responsible for teaching an entire lab, under the close observation of a DNALC staff member.

DNA *Sequencing Service*

A unique aspect of the DNALC's educational portfolio is the free DNA *Sequencing Service* and bioinformatics tools that allow students to analyze a 440-nucleotide portion of their own mitochondrial chromosome. The mitochondrion, the organelle that produces energy for the cell, contains several copies of its own genome; moreover, there are hundreds to thousands of mitochondria per cell. This amplification and the fact that the noncoding (control) region of the mitochondrial genome accumulates mutations quickly make the control region ideal for studies of human evolution and diversity.

Using a kit distributed by Carolina Biological Supply Company, students isolate DNA from cheek cells and then use PCR to amplify the mitochondrial control region. The student samples are then sent by overnight mail to the DNALC, where they are prepared for sequencing by college interns Alina Duvall (Hofstra University) and Jennifer Aiello (Long Island University, C.W. Post Campus). The student samples then go to the CSHL Sequencing Shared Resource Facility in Woodbury, where they are sequenced on an Applied Biosystems 3730xi Genetic Analyzer. Finally, the sequences are uploaded to the *Sequence Server* database at our *BioServers* internet site (http://www.bioservers.org). There, students can view their sequences and use software to align them with mitochondrial sequences from modern humans, ancient humans, primates, and other animals. In this way, they can use their own DNA as a starting point to discover what DNA says about how humans evolved and migrated around the globe.

In 2008, we sequenced 7003 student DNA samples—a 10% increase from 2007. Samples were received from 145 high schools, 36 community colleges, and 71 universities. On average, results were posted on the *BioServers* site within 20 days of receipt. The free service is made possible by the donation of sequencing reagents by Applied Biosystems of Foster City, California.

Staff and Interns

In August, we said goodbye to high school instructor Brian Lang and middle school instructor Lauren Weidler. We will miss them both and wish them continued success as they teach biology—Brian in the North Shore School District and Lauren in the Plainview School District. Instructional vacancies were filled by Jason Williams and Tedi Setton, two Long Island natives. Jason has a bachelor's degree in biology from

SUNY Stony Brook. He worked at CSHL's main campus, involved in the molecular genetics of plant development in *Arabidopsis* and cancer research. Tedi brings a wealth of knowledge from her undergraduate degree in biological sciences from Wellesley College and her studies in forensic pathology at the Suffolk County Medical Examiner's Office. Having participated in a *DNA Science* summer workshop as a high school student, Tedi's education came full circle when she led *DNA Science* as an instructor!

In the fall, we were lucky to recruit Dr. Ileana Rios to head our new *Harlem DNA Lab*. Ileana brings an informed perspective to our effort to bring high-level science to underrepresented students in New York City. Born in Spanish Harlem, only a few blocks from the Harlem Lab site, she went on to receive a B.A. in biology from Barnard, a B.S. in cytology from SUNY Health Science Center, and a Ph.D. in molecular biology from CUNY. Her work history spans research in cellular senescence and HIV vaccine development, as well as teaching at the elementary, secondary, and undergraduate levels.

We also welcomed back Dr. Uwe Hilgert after a year's assignment as Assistant Dean of the Watson School of Biological Sciences, where he eased a transition between Deans. Uwe returned to spearhead the DNALC component of the *iPlant* Collaborative and to resume his role as scheduling tsar, maintaining our increasingly complex schedule of teacher training workshops. As a microbiologist, Uwe brings to the *iPlant* position formal training in plant pathology/physiology from the Max-Planck Institute for Plant Breeding and the University of Arizona. He has practical expertise in bioinformatics education, having worked on high school and teacher training under HHMI and NSF grants. He had a key role in developing the DNALC's *Dynamic Gene* internet site, an interface that allows students to annotate the rice genome sequence. He also knows many of the players in the *iPlant* leadership, having spent his postdoctoral period at the University of Arizona before coming to CSHL in 2000.

Lauren Correri, our new Administrative Assistant, is a native Long Islander. After graduating from Adelphi University and working toward a master's degree at Sarah Lawrence College, she gained administrative experience at First Advantage, a firm specializing in insurance investigations. Her duties at the DNALC include greeting visitors, overseeing scheduling, collecting fees, and tracking visitation.

The internship program continues to draw some of Long Island's most talented high school and college students, engaging them in science research and providing practical laboratory experience. We were pleased to accept a large group of new high school interns this year: Dylan Assael (Syosset), Michelle Bobrow (Great Neck South), Nancy Desai (Hicksville), Utkarsh Sahay (North Shore), Yasmina Macer (Portledge), Andrew Malmgren (Massapequa), Anastasia Minkin (Oyster Bay), Emily Troge (Our Lady of Mercy), Kaitlin Watrud (Commack), and Kevin Wu (Jericho). Returning interns were Charmaine Brown (Westbury), Rachel Gellerman (Cold Spring Harbor), Stephanie Parascandolo (Half Hollow Hills), and Arielle Scardino (Kings Park). Many of our interns continued to invest their DNALC experience in their own research. Arielle

New staff at the DNALC in 2008 (*left to right*): Lauren Correri, Tedi Setton (*standing*), Ileana Rios, and Jason Williams

Scardino (Kings Park) worked with Jermel Watkins to explore how a decreased expression of certain mitochondrial genes can be used as a biomarker for neuromuscular dysfunction.

We bid farewell to interns Seth Schortz, who started at Emory University, and Benjamin Blond, who started at Yale University. Former high school interns Nick Wilkin (Ithaca College) and Janice Yong (Boston University) returned to help out during college breaks. Lauren Thompson (Barnard College) again returned to assist with summer workshops at DNALC *West*.

College intern Jennifer Aiello (C.W. Post University) continued to effectively manage our free DNA *Sequencing Service*. She also called on her major in forensic science to help us create a popular family workshop for *Saturday DNA*, *Dig This!*, a hands-on introduction to forensic anthropology.

Expert Advisors and Corporate Support

We are lucky to have high-level support from two advisory bodies: the Education Committee and the Corporate Advisory Board (CAB). The Education Committee formulates policy and assists with strategic planning, whereas the CAB provides a liaison to the Long Island business community. The CAB conducted its annual fund campaign and golf tournament, raising $275,000.

DNALC Committee
Chairperson: **Laurie Landeau, V.M.D.**

Lori Bressler
Edward A. Chernoff
Lola N. Grace
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Suzanne Leeds
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Lee Shuett, Nikon Instruments
Kurt Timmel, Marsh USA
Jeffrey K. Tupper, U.S. Trust Company of New York
Robert Van Nostrand, AGI Dermatics
David Widmer, Long Island Radio Group
Hans Zobel, Ziehm Medical LLC

David A. Micklos
Executive Director

2008 Workshops, Meetings, and Collaborations

January 4	Site visit by Debra Berhans, Canisius College, Buffalo, New York
January 7	Site visit by Leo Brizuela, Agilent Technologies, Inc., Melville, New York
January 10	Watson School Orientation and Welcome Reception, DNALC
January 12	*Saturday DNA!*, "Survival of the Fittest" and "Blame it on the Brain," DNALC
January 14	Meeting with Steve Barkanic, Gates Foundation, Washington, D.C.
January 16	Site visit by William Fair, Juliette Morgan, and Connie Hildesley, Alexandria Real Estate Equities, New York
January 17	Site visit by Scott Bronson, Mel Morris, and Ken White, Brookhaven National Laboratory, Upton, New York
January 18–19	NSF *Dynamic Gene* Workshop, Salk Institute for Biological Studies, San Diego, California
January 26	American Museum of Natural History Workshop, "Discovering the Tree of Life," New York
February 5	DNALC Education Committee Meeting, DNALC
February 6	Site visit by David Widmer, Long Island Radio Group, Farmingdale, New York
February 6	Site visit by Lorraine Aycock and Monica Cavallo, Bank of America, Melville, New York
February 7	Site visit by Katherine Charleston Colgate, Callisto Pharmaceuticals, New York
February 7–8	BEN Collaborators Meeting, AAAS Headquarters, Washington, D.C.
February 9	*Saturday DNA!*, "Superbugs Uncovered" and "Immuno Exploration," DNALC
February 11	Site visit by *G2C Online* Fellow Laura Maitland, AP psychology Consultant
February 19	Site visit by *G2C Online* Fellow Laura Maitland, AP psychology Consultant
February 21	Site visit by *G2C Online* Fellow Caren Gough, Education Consultant
February 27	Human DNA Fingerprinting Laboratory, members of DNALC Corporate Advisory Board, DNALC
March 5	Science Fair at John S. Roberts Educational Complex, East Harlem, New York
March 5	PBS filming of DNALC instructors for documentary, WLIW Teaching and Learning Celebration, *Harlem DNA Lab*, East Harlem, New York
March 5	Site visit by Marilyn Simons and staff, Simons Foundation, New York
March 6	Site visit by Gail Bennington, Garden City Science Curriculum Coordinator; Adele Cacotti, AP Teacher, Garden City High School; Steve Gordon, Science Research Teacher; and 25 Singaporean science research students
March 7	Meeting with Bill Fair and Connie Hildesley, Alexandria Real Estate Equities, New York
March 7–8	WNYC and WLIW Teaching and Learning Celebration, New York
March 11	Meeting with Phillip Griffiths, Carnegie Corporation of New York and Institute for Advanced Study Commission on Math and Science Education, New York
March 12–13	Hewlett Foundation Grantee Meeting, Carnegie Mellon University, Pittsburg, Pennsylvania
March 13	New York Department of Education Science Leadership Team Meeting, CUNY, New York
March 15	*Saturday DNA!*, "Living in the Gene Age: Chronicles & Frontiers" and "Gram-*tastic*!," DNALC
March 19	Presentation for Science Supervisors' Association of New York City, American Museum of Natural History, New York
March 19	Meeting with Richard Kahan, Urban Assembly, and Mort Slater, Gateway Institute for Higher Education, New York
March 19	Meeting with Jose Maldonado and Sam Silverstein, Columbia Secondary School for Math, Science, and Engineering, New York
March 19	Site visit by Babylon High School science faculty
March 21	Site visit by *G2C Online* Fellow Caren Gough, Education Consultant
March 21	Site visit by Bob Wheeler, Pine Creek High School, Colorado Springs, Colorado, and Brenda Dempsey, Castle View High School, Castle Rock, Colorado
March 21–22	NSF *Plant Molecular Genetics and Genomics* Follow-up Workshop, Florida Agricultural & Mechanical University, Tallahassee, Florida
March 25	Westbury school district teacher training, DNALC
March 26	Site visit by Alister Jones and Bronwen Cowie, School of Education, University of Waikato, New Zealand
March 27	Open House, *Harlem DNA Lab*, East Harlem, New York
March 27–29	National Science Teachers Association Workshops, Boston, Massachusetts
April 7–9	NSF *iPlant* Collaborative interviews at the *iPlant* Inaugural Conference: *Bringing Plant and Computing Scientists Together to Solve Grand Challenges,* CSHL
April 7–June 13	Internship by Amy Niselle, graduate student, Melbourne, Australia, *DNA Interactive* and *G2C Online,* DNALC
April 11	NIH *Inside Cancer* Workshop, Biolink Southwest Regional Meeting, Albuquerque, New Mexico
April 12	*Saturday DNA!*, "The Mystery of Anastasia Romanov" and "Inside Cancer," DNALC
April 12	Science Council of New York City Conference, Stuyvesant High School, Brooklyn, New York
April 15	Open House, *Harlem DNA Lab*, East Harlem, New York
April 14–16	Westbury school district teacher training, Westbury, New York
April 17	*G2C Online* Interview with Josh Dubnau, CSHL
April 18	Site visit by Lucio Pinto, Director, Tronchetti Foundation, Milan, Italy
April 22	Wellcome Trust Sanger Institute review of National Science Learning Center, York, United Kingdom
April 24	Site visit to Wellcome Trust Sanger Institute, Cambridge, United Kingdom
April 25	Site visit by *Long Island Pulse* magazine science fantasy contest winner, Gloria Schramm
April 29	*Great Moments in DNA Science* Honors Seminar: "Mutate Me Once, Permit a Tumor; Mutate Me Twice, Inhibit," Lloyd Trotman, CSHL
May 1	Site visit by Hisao Izawa, President, Nikon Instruments Worldwide; Lee Shuett, CAB Board Member, Executive VP Nikon; and Yoichi Kasahara, Planning General Manager, Nikon
May 8	Site visit by Ann McDermott-Kave, Director, OSI Foundation; Morgan Browne, Chairman, OSI Foundation; and David Epstein, DNALC CAB member and OSI vice president of oncology
May 8	*G2C Online* Interview with Josh Dubnau, CSHL

May 8–9 NSF *Dynamic Gene* Workshop in Gene Annotation, *Harlem DNA Lab*, East Harlem, New York
May 10 *Saturday DNA!*, "Survival of the Fittest" and "A Billion Little Pieces," DNALC
May 13 *Great Moments in DNA Science* Honors Seminar: "Protein Demolition: Taking Control by Taking Apart," Bill Tansey, CSHL
May 17 Oyster Bay Health and Science Fair, Oyster Bay, New York
May 19–21 NIH SEPA Principal Investigators Meeting, Rockville, Maryland
May 27 *Great Moments in DNA Science* Honors Seminar: "Genotype to Phenotype: A Closer Look at Plant Genomics," by Doreen Ware, CSHL
May 27 Site visit by Amy Sanders, Project Manager, Darwin 200, Wellcome Trust
May 28 Site visit by Vinford Mentar, JP Morgan Chase Global Philanthropy, New York
June 2–6 NSF *Silencing Genome*s Workshop, Rust College, Holly Springs, Mississippi
June 4 Site visit by Grey Ruegamer, New York Giants football team
June 7 Howard Hughes Medical Institute Fellows Meeting, DNALC
June 9 Site visit by Kidgie Williams, Hospitality Committee for United Nations Delegations, Inc., and United Nations delegates' family members, New York
June 9–13 Site visit by *G2C Online* Fellow Caren Gough, Education Consultant
June 11 Site visit by Rut Premsrirut, CSHL, and Michael Rialdy, Indonesia
June 12 Site visit by *G2C Online* Fellow Laura Maitland, AP psychology Consultant
June 12 Site visit by Tom Burrish, Provost, University of Notre Dame; William Doyle, Sr., Director of Development; Tom Gibbons, Regional Director of Development, New York Metro; and John Passarelli, Notre Dame Alumni and Board Member, DNALC
June 14 *Saturday DNA!*, "Cancer: Inside Out" and "Human Evolution and mtDNA," DNALC
June 17 Fifteenth Annual Golf Tournament, Piping Rock Country Club, Locust Valley, New York
June 20–21 NSF *Plant Genetics and Genomics* Follow-up Workshop, Langston, Oklahoma
June 26 Site visit by Alfred Goldberg, Professor, Harvard Medical School and former CSHL URP (1961), of the Porter Foundation
June 27–July 2 American Association of Plant Biologists, Plant Biology 2008, Mérida, Mexico
June 30–July 3 *Fun with DNA* Workshop, DNALC
Fun with DNA Workshop, DNALC *West*
Fun with DNA Workshop, Central Islip
World of Enzymes Workshop, DNALC
Plant Genomics Workshop, DNALC
July 1 Site visit by Miriam Cortes-Caminero, Director of Marketing, Laboratory Technologies, Inc., Maple Park, Illinois
July 2–8 *G2C Online* Interviews with Jeff Lichtman, Harvard University; Ken Kosic, University of California, Santa Barbara; James Eberwine, University of Pennsylvania; Rusty Lansford, California Institute of Technology; and Pierre Marie Lledo, Pasteur Institute, Paris, France, CSHL
July 7–11 *Fun with DNA* Workshop, DNALC
Green Genes Workshop, DNALC
DNA Science Workshop, DNALC
DNA Science Workshop, *Harlem DNA Lab*
World of Enzymes Workshop, DNALC *West*
Genomic Biology Workshop, Central Islip
July 11–15 International Congress of Genetics, Berlin, Germany
July 14 *G2C Online* Interviews with Anil Malhotra, North Shore Long Island Jewish Hospital, and David Skuse, University College, London, England, CSHL
July 14–18 *World of Enzymes* Workshop, DNALC
Genetic Horizons Workshop, DNALC
DNA Science Workshop, DNALC *West*
July 14–August 1 *Amgen Leadership Symposium*, DNALC
July 15 *G2C Online* Interview with Sarah Blakemore, University College, London, United Kingdom, CSHL
July 17 Site visit by Molloy College students, Pat Mason, Group Leader
July 17 Site visit by Coleman Fung, Founder, Open Link Financial Inc., Uniondale, New York
July 17–18 *G2C Online* Interviews with Allen Moore, University of Exeter, England; Christian Keysers, BCN Neuroimaging Center, Groningen, The Netherlands; and Larry Young, Emory University, Atlanta, Georgia, CSHL
July 19 *G2C Online* Interview with Thomas Insel, National Institute of Mental Health, Bethesda, Maryland
July 21–25 *Fun with DNA* Workshop, DNALC
Human Genomics Workshop, DNALC
DNA Science Workshop, United Way/Project Grad with Westbury Public Schools, Hofstra University, Westbury, New York
Green Genes Workshop, DNALC *West*
DNA Science Workshop, Central Islip, New York
July 24 *G2C Online* Interview with Wayne Drevets, National Institute of Mental Health, Bethesda, Maryland
July 24 *G2C Online* Interviews with Kay Redfield Jamison and James Potash, Johns Hopkins University, Baltimore, Maryland
July 25 *G2C Online* Interviews with Johan Jansma, Richard Coppola, and Wayne Drevets, National Institute of Mental Health, Bethesda, Maryland
July 28–August 1 *Fun with DNA* Workshop, DNALC *West*
World of Enzymes Workshop, DNALC
Green Genes Workshop, DNALC

August 4–8	*Fun with DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
	Genetic Horizons Workshop, DNALC
	DNA Science Workshop, DNALC
August 4–8	Site visit by HHMI Fellows Caren Gough, Jerry Watkins, Greg Borman, Dahlia McGregor, Kathleen Rucker, and Adrienne Rubin for Harlem Professional Development
August 4–8	NSF *Silencing Genomes* Workshop, Fralin Biotechnology Center, Virginia Tech, Blacksburg, Virginia
August 7	Site visit by Zaven Khachaturian, Ph.D., President and CEO, and Ara Khachaturian, Ph.D., Chief Program Officer, Lou Ruvo Brain Institute & Keep Memory Alive, Las Vegas, Nevada
August 7	Site visit by 17 math and science teachers, The New York Center for Teacher Development, Inc.
August 11–15	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNALC
	DNA Science Workshop, DNALC *West*
	Excelsior Scholars Workshop, *Harlem DNA Lab*
August 13–15	NSF Course, Curriculum, and Laboratory Improvement Program, Principal Investigators Conference, Washington, D.C.
August 15	*G2C Online* Presentation, American Psychological Association, Boston, Massachusetts
August 18–22	*Fun with DNA* Workshop, DNALC
	Genetic Horizons workshop, DNALC
	DNA Science Workshop, DNALC
	Human Genomics Workshop, DNALC *West*
	Excelsior Scholars Workshop, *Harlem DNA Lab*
August 18–22	NSF *Silencing Genomes* Workshop, Biolink Texas (Austin Community College), Austin, Texas
August 22	Site visit by Taiwan Delegation: Fuh-Sheng Shieu, Huann-Shyang Lin, Sue-Joan Chang, Horn-Jiunn Sheen, Ching-Yang Chou, Yu-Chie Wang, Tzong-Ming Wu, Sung-Tao Li, Ching-Mei Tang, Yen-Hung Pan, and Ching-Jiun Lee
August 25–29	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
	Genetic Horizons Workshop, DNALC *West*
	DNA Science Workshop, DNALC
	DNA Science Workshop, *Harlem DNA Lab*
August 26	Site visit by Adele Smithers-Fornaci, President, The Christopher D. Smithers Foundation, Inc., Mill Neck, New York
August 29	Site visit by Richard Gelfond, President of IMAX; Pamela Gelfond, Yale graduate; and Claudia Gelfond, Dalton School graduate
August 29	Site visit by William Mak, Hong Kong Biotechnology Education Resource Center, Hong Kong University
September 4–5	NSF Review, Research Coordination Network, Arlington, Virginia
September 14–15	Site visit to Genome Sequencing Center, Washington University, St. Louis, Missouri
September 17–18	Site visit by Martha Narro and Lisa Howells, NSF *iPlant* Collaborative
September 18–19	*G2C Online* Workshop, Stowers Institute for Medical Research, Kansas City, Missouri
September 20	NIH *Inside Cancer* Workshop, Stowers Institute for Medical Research, Kansas City, Missouri
September 23	Dedication of *Harlem DNA Lab*, East Harlem, New York
September 24	*G2C Online* Interviews with Ellen Leibenluft, Danny Pine, Judy Rapaport, and Phil Shaw, National Institute of Mental Health, Bethesda, Maryland
September 25	Site visit and presentation of grant award by Lorraine Aycock, Vice President, Market Development Manager, and Monica Cavallo, Market Development Specialist, Bank of America of LI
September 25	Presentation of *Harlem DNA Lab* programs, NYC Department of Education's *Citywide Science Leadership Team*, Wave Hill, Bronx, New York
September 26	Site visit by *G2C Online* Teaching Fellow Bob Keltos
September 27	HHMI Professional Development Program for NYC DOE Teachers, seminar on "DNA Structure and Isolation," *Harlem DNA Lab*, East Harlem, New York
September 30–October 3	NSF *iPlant* Collaborative Grand Challenge Workshops *Mechanistic Basis of Plant Adaptation* and *Impact of Climate Change on Plant Productivity* Biosphere 2, University of Arizona, Tucson
October 3–4	*G2C Online* Workshop, DNALC
October 4	HHMI Professional Development seminar "Transformation and Protein Isolation," *Harlem DNA Lab*, East Harlem, New York
October 8	Cold Spring Harbor High School Partnership Program commences
October 9	Public School meeting, McMain Secondary School, New Orleans, Louisiana
October 10–11	*G2C Online* Fellows Meeting, DNALC
October 14	HHMI Professional Development seminar, "Transformation and Protein Isolation," *Harlem DNA Lab*, East Harlem, New York
October 15	NIH *Inside Cancer* Workshop, National Association of Biology Teachers Professional Development Conference, Memphis, Tennessee
October 16	Site visit by Piero Beneditti and 18 students, Natural Science Museum, Sardinia, Italy
October 16	*G2C Online* Presentation, National Association of Biology Teachers Professional Development Conference, Memphis, Tennessee
October 18	NIH *Inside Cancer* Workshop, Midland College, Midland, Texas
October 24	Site visit and tour for Dorota Koczewska, Director of Mathematics and Science, District 75/Citywide Program, New York

October 25	HHMI Professional Development Seminar, "DNA Analysis and Forensics," *Harlem DNA Lab*, East Harlem, New York
October 25	*Saturday DNA!*, "How to Solve a Crime," DNALC
October 29	NY1 Interview by health reporter Kafi Drexel of Emily Carey School of students visiting *Harlem DNA Lab*, East Harlem, New York
October 31–November 1	*G2C Online* Workshop, Fred Hutchinson Cancer Research Center, Seattle, Washington
November 1	Dedication of *The Laurie J. Landeau Multimedia Studio* and DNALC 20th Anniversary Celebration, DNALC
November 2	NIH *Inside Cancer* Workshop, Fred Hutchinson Cancer Research Center, Seattle, Washington
November 4	HHMI Professional Development Seminar, "Using Genetics to Engage and Accelerate Student Learners," *Harlem DNA Lab*, East Harlem, New York
November 6	Site visit by Caren Gough, Teaching Fellow, *G2C Online*
November 7	NIH *Inside Cancer* Workshop, Kimmel Cancer Center, Philadelphia, Pennsylvania
November 7–8	Forensic DNA Workshop, for Biology Teachers of New Jersey, NABT, DNALC
November 7–10	NSF *iPlant* Collaborative Grand Challenge Workshop, *Developing Common Mechanistic Models for the Plant Sciences*, Biosphere 2, University of Arizona, Tucson
November 8	HHMI Professional Development Seminar, "Variability & Inheritance," *Harlem DNA Lab*, East Harlem, New York
November 10	HHMI Professional Development Seminar, "DNA Analysis & Forensics," *Harlem DNA Lab*, East Harlem, New York
November 14	HHMI Professional Development Seminar, "PCR and Human DNA Variations, Part One," *Harlem DNA Lab*, East Harlem, New York
November 14–15	*G2C Online* Workshop, St. Louis Science Center, St. Louis, Missouri
November 15	*Saturday DNA!*, "GMO IQ," DNALC
November 16–18	*G2C Online* Interviews with Rene Anand, Ohio University; Brian Bacskai, Harvard University; Randy Blakely, Vanderbilt University; Kalanit Grill-Spector, Stanford University; Todd Sacktor, State University of New York Downstate Medical Center, Brooklyn; Donna Wilcock, Duke University; Abraham Zangen, Weismann Institute of Science, Israel; Tallie Z. Baram and Nicole Gage, University of California, Irvine; John J. Mann, Columbia University; Lisa Monteggia, University of Texas Southwestern Medical Center; Dennis Selkoe, Harvard University; Gul Dolen, Massachusetts Institute of Technology; Helen Myberg, Emory University; Bruce McEwen, The Rockefeller University; and Simona Spinelli, National Institute of Health, at The Society for Neuroscience Annual Meeting, Washington, D.C.
November 19–23	NSF *iPlant* Collaborative Grand Challenge Workshop, *Assembling the Tree of Life to Enable the Plant Sciences,* Biosphere 2, University of Arizona, Tucson
November 20	*G2C Online* Presentation, National Science Teachers Association Meeting, Portland, Oregon
November 22	HHMI Professional Development Seminar, "PCR and Human DNA Variations, Part One," *Harlem DNA Lab*, East Harlem, New York
November 24	Team Temasel, Singapore, hosted by Briarcliff Manor High School, *Harlem DNA Lab*, East Harlem, New York
November 30–December 13	Singapore Primary Teachers Attachment, DNALC
December 1	*G2C Online* Advisory Panel Meeting, DNALC
December 4	*G2C Online* Presentation, National Science Teachers Association Meeting, Cincinnati, Ohio
December 4	Site visit by Andrew Tasker, Champalimaud Foundation, Lisbon, Portugal
December 6	HHMI Professional Development Seminar, "Transformation and Protein Isolation," *Harlem DNA Lab*, East Harlem, New York
December 8	HHMI Professional Development Seminar, "PCR and Human DNA Variations, Part Two," *Harlem DNA Lab*, East Harlem, New York
December 8	Site visit by Belgian Delegation: Geert Schelstraete, Jo Decuyper, Sofie Stoop, Jo De Wachter, Imke Debecker, Ann Van Gysel, and Jan Wauters
December 8	Cold Spring Harbor High School Partnership Program Student Debate, DNALC
December 10	Northern Manhattan Coalition for Math and Science, *Harlem DNA Lab*, East Harlem, New York
December 12	HHMI Professional Development Seminar, "Engaging Level One and Level Two Student Learners," *Harlem DNA Lab*, East Harlem, New York
December 13	*Saturday DNA!*, "Dig This," DNALC
December 15–17	NSF *iPlant* Collaborative Grand Challenge Workshop, *Computational Morphodynamics*, Biosphere 2, University of Arizona, Tucson
December 15	Site visit by Mike Scanlon, Cornell University, Ithaca, New York
December 18	NYC DOE Science Specialists, *Harlem DNA Lab*, East Harlem, New York
December 20	HHMI Professional Development Seminar, "DNA Analysis and Forensics," *Harlem DNA Lab*, East Harlem, New York
December 30	Site visit by NAACP ACT-SO (Afro-Academic, Cultural, Technological, and Scientific Olympics)

Sites of Major Faculty Workshops 1985–2007

Key: *Middle School* High School **College**

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**
	California Institute of Technology, Pasadena	**2007**
	Canada College, Redwood City	1997
	City College of San Francisco	2006
	Contra Costa County Office of Education, Pleasant Hill	2002
	Foothill College, Los Altos Hills	**1997**
	Harbor-UCLA Research & Education Institute, Torrance	2003
	Los Angeles Biomedical Research Institute (LA BioMed), Torrance	**2006**
	Laney College, Oakland	1999
	Lutheran University, Thousand Oaks	1999
	Pierce College, Los Angeles	**1998**
	Salk Institute for Biological Studies, La Jolla	2001, 2008
	San Francisco State University	**1991**
	San Jose State University	**2005**
	University of California, Davis	1986
	University of California, Northridge	**1993**
COLORADO	Aspen Science Center	2006
	Colorado College, Colorado Springs	1994, 2007
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
FLORIDA	Armwood Senior High School, Tampa	1991
	Florida Agricultural & Mechanical University, Tallahassee	2007–2008
	North Miami Beach Senior High School	1991
	University of Miami School of Medicine	2000
	University of Western Florida, Pensacola	1991
GEORGIA	Fernbank Science Center, Atlanta	1989, 2007
	Morehouse College, Atlanta	**1991, 1996–1997**
HAWAII	Kamehameha Secondary School, Honolulu	1990
IDAHO	University of Idaho, Moscow	1994
ILLINOIS	Argonne National Laboratory	1986–1987
	University of Chicago	**1992, 1997**
INDIANA	Butler University, Indianapolis	1987
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
	National Center for Biotechnology Information, Bethesda	2002
	St. John's College, Annapolis	*1991*
	University of Maryland, School of Medicine, Baltimore	**1999**
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
	The Winsor School, Boston	1987
	Whitehead Institute for Biomedical Research, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
MINNESOTA	University of Minnesota, St. Paul	2005
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990–1991
	Rust College, Holly Springs	2006–2008
MISSOURI	St. Louis Science Center, St. Louis	2008
	Stowers Institute for Medical Research, Kansas City	2002, 2008
	Washington University, St. Louis	**1989, 1997**
NEVADA	University of Nevada, Reno	1992
NEW HAMPSHIRE	**New Hampshire Community Technical College, Portsmouth**	**1999**
	St. Paul's School, Concord	1986–1987
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
NEW MEXICO	Biolink Southwest Regional Meeting, Albuquerque	2008

NEW YORK	Albany High School	1987
	American Museum of Natural History, New York	2007
	Bronx High School of Science	1987
	Canisius College, Buffalo	2007
	Cold Spring Harbor High School	1985, 1987
	Columbia University, New York	**1993**
	Cornell University, Ithaca	**2005**
	DeWitt Middle School, Ithaca	*1991, 1993*
	DNA Learning Center	1988–1995, 2001–2004, 2006–2008
	DNA Learning Center	**1990, 1992, 1995, 2000**
	DNA Learning Center	*1990–1992*
	DNA Learning Center *West*	2005
	Fostertown School, Newburgh	*1991*
	Harlem DNA Lab, East Harlem	2008
	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
	New York City Department of Education	2007
	New York Institute of Technology, New York	2006
	New York Institute of Technology, New York	**2006**
	Orchard Park Junior High School	*1991*
	Plainview-Old Bethpage Middle School	*1991*
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Stuyvesant High School, New York	1998–1999
	The Rockefeller University, New York	2003
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Trudeau Institute, Saranac Lake	2001
	Union College, Schenectady	2004
	U.S. Military Academy, West Point	**1996**
	Wheatley School, Old Westbury	1985
NORTH CAROLINA	CIIT Centers for Health Research, Research Triangle Park	2003
	North Carolina Agricultural & Technical State University, Greensboro	2006–2007
	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	Langston University, Langston	2008
	North Westerville High School	1990
OKLAHOMA	**Oklahoma City Community College**	**2000**
	Oklahoma City Community College	2006–2007
	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
	Kimmel Cancer Center, Philadelphia	2008
SOUTH CAROLINA	**Clemson University, Clemson**	**2004**
	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	**1988**
TENNESSEE	NABT Professional Development Conference, Memphis	2008
TEXAS	Austin Community College–Eastview Campus, Austin	2007–2008
	Austin Community College–Rio Grande Campus	2000
	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Midland College, Midland	2008
	Southwest Foundation for Biomedical Research, San Antonio	2002
	Taft High School, San Antonio	1991
	Texas A&M, Agricultural Research and Extension Center, Weslaco	2007
	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**
	Utah Valley State College, Orem	**2007**
VERMONT	University of Vermont, Burlington	1989
VIRGINIA	Eastern Mennonite University, Harrisonburg	1996
	Jefferson School of Science, Alexandria	1987

	Mathematics and Science Center, Richmond	1990
	Mills Godwin Specialty Center, Richmond	1998
	Virginia Polytechnic Institute and State University, Blacksburg	**2005, 2008**
WASHINGTON	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001, 2008
	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
AUSTRIA	**Vienna Open Lab**	**2007**
CANADA	Red River Community College, Winnipeg, Manitoba	1989
GERMANY	Urania Science Center, Berlin	2008
ITALY	Porto Conte Research and Training Laboratories, Alghero	1993
	International Institute of Genetics and Biophysics, Naples	1996
MEXICO	ASPB Plant Biology, Mérida	2008
PANAMA	**University of Panama, Panama City**	**1994**
PUERTO RICO	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SINGAPORE	National Institute of Education	2001–2005
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	
	Uppsala University, Uppsala	2000

COLD SPRING HARBOR LABORATORY PRESS

2008 PUBLICATIONS

SERIALS

Genes & Development, Vol. 22, 1–33520 (www.genesdev.org)
Genome Research, Vol. 18, 1–2044 (www.genome.org)
Learning & Memory, Vol. 15, 1–920 (www.learnmem.org)
RNA, Vol. 14, 1–2712 (www.rnajournal.org)
Cold Spring Harbor Symposia on Quantitative Biology, Vol. 72: *Clocks and Rhythms,* Bruce Stillman, David Stewart, and Terri Grodzicker (eds.)
Cold Spring Harbor Protocols (www.cshprotocols.org)

LABORATORY MANUALS

Basic Methods in Protein Purification and Analysis, edited by Richard J. Simpson, Peter D. Adams, and Erica A. Golemis
Emerging Model Organisms: A Laboratory Manual, Volume 1
Proteomics: A Cold Spring Harbor Laboratory Course Manual, Andrew J. Link, Philip Andrews, and Joshua LaBaer
Transcriptional Regulation in Eukaryotes: Concepts, Strategies, and Techniques, Second Edition, Michael Carey, Stephen Smale, and Craig Peterson

HANDBOOKS

C. elegans *Atlas,* David H. Hall and Zeynep F. Altun
A Short Guide to the Human Genome, Stewart Scherer

TEXTBOOKS

Essentials of Glycobiology, Second Edition, edited by Ajit Varki, Richard Cummings, Jeffrey Esko, Hudson Freeze, Pamela Stanley, Carolyn Bertozzi, Gerald Hart, and Marilynn Etzler

SCIENCE AND SOCIETY

Concerning the Origins of Malignant Tumours, Theodor Boveri, translated and annotated by Sir Henry Harris
Experimental Heart: A Novel, Jennifer Rohn
Davenport's Dream: 21st Century Reflections on Heredity and Eugenics, edited by Jan A. Witkowski and John R. Inglis
Grounds for Knowledge: A Guide to Cold Spring Harbor Laboratory's Landscapes and Buildings, Introducing the Bungtown Botanical Garden, Elizabeth L. Watson, landscape photography by Peter Stahl
Life Illuminated: Selected Papers from Cold Spring Harbor, Volume 2, 1972–1994, edited by Jan Witkowski, Alexander Gann, and Joseph Sambrook
My Heart vs. the Real World: Children with Heart Disease, In Photographs & Interviews, Max Gerber
Neither Gods Nor Beasts: How Science Is Changing Who We Think We Are, Elof A. Carlson
The Strongest Boy in the World: How Genetic Information Is Reshaping Our Lives, Updated and Expanded, Philip R. Reilly

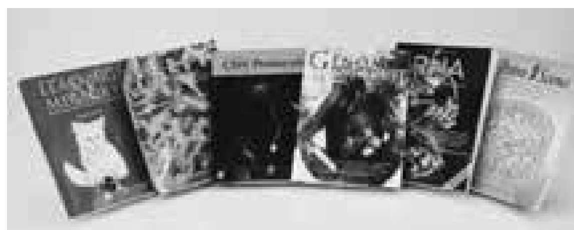
OTHER

CSHL Annual Report 2007: Yearbook Edition
CSHL Annual Report 2007
Banbury Center Annual Report 2007
Watson School of Biological Sciences Annual Report 2007

WEBSITES

www.cshteaching.org (formerly www.genesandsignals.org)
http://www.evolution-textbook.org
http://www.cshl.symposium.org; *Symposia*, Vol. 72 added to online *Symposia* website

A selection of recently published books



The journal publishing program

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

In 2008, despite challenging industry conditions, Cold Spring Harbor Laboratory Press generated an operating excess of $597,000 on revenues of $11 million. The Press' total financial contribution to the Laboratory for the year was more than $1.2 million. The Press also continued to fulfill its mission of publishing print and electronic media of exceptional quality and value to the scientific community.

Journals and Online Publications

Revenue from our journals publishing program was more than 11% higher than in 2007. New institutional journal subscriptions were gained despite a tightening market, and in the case of *CSH Protocols*, our newest journal, the total institutional subscription base more than doubled. International consortia sales increased, via agreements for representation in Latin America, Australia, and New Zealand. Subscription trials and negotiations with consortia are currently in progress in Brazil, India, and Germany. Recently developed databases of global journal subscription records are being used to examine our journals' potential for further growth.

Impact factor analysis continues to rank our journals *Genes & Development* and *Genome Research* at the top of their disciplines. *Genome Research* reached a new high of 11,224. In 2008, both these journals, as well as *RNA* and *Learning & Memory*, received more offers of manuscripts to publish than in any previous year, placing a premium on the careful editorial selection of papers for publication. New tools are being used to analyze the metrics of journal value, such as the impact and the Eigen factors, that are influenced by the usage of individual papers and article types.

Regular press releases ensured that print, online, and broadcast media attention was drawn to research findings of broad public interest published in *Genes & Development* and *Genome Research*.

A major upgrade of the online editions of our journals was completed in October. Hosted on Stanford University's new "H2O" platform, CSHL Press journals are now able to interact with other online systems and to be extended with emerging web services and technologies. One publication that has been transformed by this upgrade is the online edition of the *Annual Symposia on Quantitative Biology*, which was relaunched in a new, improved website that combines the proceedings of the meetings as published papers with author interviews, photographs, and video sequences. The print volume will continue to be published. A complete electronic archive of the first 70 symposia (1933–2004), including many classic volumes now rarely available in print, was released in November 2008. Initial sales were strong and reached more than $45,000 in the first three months.

Development of the new journal *CSH Perspectives* continued, with a launch set for July 2009. This new online-only publication will offer rapid access to collections of articles commissioned around single topics of broad scientific interest, with print publications following as topic-specific collections are completed.

A rebranding of the CSHL Press website is underway to promote the theme "Support Research and Save." Text and graphics will highlight discount prices available to our web customers and detail how a portion of each purchase contributes to the Lab's research and scientific activities.

Book Division, Cold Spring Harbor Laboratory Press

Our website continues to add new features, including a Google book search tool that allows full-text searching within most of our book titles. Search results provide limited page views along with links to web-based information related to the book, such as citations, news articles, reviews, and blogs. Improvements to the Press website also included the addition of new shopping cart logic to enable more effective price promotions via our sale section, monthly newsletter, and special campaigns.

Initial evaluation of an additional solution for online publishing, with much lower costs, has shown potential and will be implemented in 2009, initially to bring to market a 50-volume archive of the classic Cold Spring Harbor Monograph series, begun in 1972. The goal of this effort is to provide the Press with the capability of publishing new online books and unique electronic media products with lower risk and better return on the investment per title.

Books

Sixteen new books were published this year, consisting of laboratory manuals, handbooks, monographs, and history/biographies. The list was generally strong but *A Short Guide to the Human Genome*, by Stewart Scherer, constructed as a question-and-answer dialogue, was particularly well received. As a new initiative in engaging the public with science, the Press published a novel entitled *Experimental Heart* by Jennifer Rohn that portrayed science and scientists in such a real and therefore unusual way in fiction, that the book was warmly reviewed in major scientific journals.

Liz Watson at a recent book signing for *Grounds for Knowledge*

We are also pleased to publish Elizabeth Watson's beautifully designed and illustrated guide to the landscape of the Laboratory, *Grounds for Knowledge*.

Evolution, the textbook published in June 2007, continued to gain class adoptions in America, Australia, Africa, and Europe, as well as excellent feedback from professors. The companion website is now a rich bank of ancillary material and additional chapters.

During the year, a marketplace for electronic books in science finally emerged, aided by the new Kindle publishing platform that allows content to be read on the platform's dedicated reader or other devices, notably the iPhone. The first five Cold Spring Harbor titles are now available as Kindle editions.

A total of 21 new book publishing contracts were signed in 2008, with a particular focus on techniques and topic collections that will be published both in print and on the web. The other commissioning focus, laboratory handbooks, will be delivered as both print and downloadable electronic editions.

Collaboration with international publishers and book distributors augments the reach of our books. In 2008, the Press appointed an agent with exclusive rights to distribute our titles in India and the Indian subcontinent, as well as a sales representative in Asia. Seven agreements were made for translation of the Press' titles into Chinese, German, Greek, Korean, and Japanese.

An Anniversary

Although the first book from Cold Spring Harbor appeared in 1934—the product of Reginald Harris' desire to extend the audience for the proceedings of the first *Symposium in Quantitative Biology*—a Press with the Laboratory's name was formally established only in 1988. The 20th anniversary year was a chance to reflect with some pleasure on the creation of prestigious journals and some of the most widely used books in the life sciences, during a period of unprecedented change in how scientific information is created, delivered, and consumed. Neither a university press nor the product of a scientific society, Cold Spring Harbor's publishing program is unique, and the prestige of its books, journals, and online media contribute immeasurably to the Laboratory's reputation in the world of science. Support and encouragement from the Laboratory's leadership has been vital during the past decade of change and continues to be needed as we navigate the opportunities ahead.

U.S. Embassy Cultural Affairs Officer Karen Grissette (*left*) donating science textbooks to Professor Paul Gwakisa of Sokoine University of Agriculture, Tanzania, Africa. Cold Spring Harbor titles were prominent among the books, which were provided via the American Society for Cell Biology to the Genomic Institute Libraries, Veterinary School, and professors at the university. (Photo courtesy of the American Embassy.)

Staff

The accomplishments of any publishing organization are determined by the skill and professionalism of those who do its work. The Press is fortunate to have staff with both qualities in abundance—and receives numerous testimonials to that fact from authors and editors.

In 2008 we welcomed new colleagues Ann Boyle as an editor-at-large and Richard Sever as a commissioning editor.

A complete list of staff members of the Press as of December 2008 is printed elsewhere in this volume. I wish to thank them all for their many and varied contributions to our enterprise, with particular gratitude to colleagues who have managerial or supervisory responsibilities: Jan Argentine, Alex Gann, Terri Grodzicker, Bill Keen, Wayne Manos, Marcie Siconolfi, Hillary Sussman, Linda Sussman, and Denise Weiss. I am also grateful to Christina Lo, who has not only improved the efficiency of the Publisher's Office but has also brought her legal training most effectively to the tasks of contracts and foreign rights management.

John R. Inglis
Executive Director
and Publisher

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2008
(with comparative financial information as of December 31, 2007)

	2008	2007
Assets:		
Cash and cash equivalents	$ 52,226,592	25,576,470
Accounts receivable	2,391,572	2,669,325
Grants receivable	8,597,399	9,037,130
Contributions receivable, net	104,681,657	67,093,000
Publications inventory	3,925,967	4,033,100
Prepaid expenses and other assets	3,599,196	4,049,972
Investments	213,424,159	333,635,052
Investment in employee residences	4,237,194	5,446,047
Restricted use assets	20,947,929	3,991,338
Deposits with bond trustee	11,886,744	29,483,468
Land, buildings and equipment, net	198,684,203	159,204,452
Total assets	$ 624,602,612	644,219,354
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 50,563,503	19,998,678
Deferred revenue	3,962,000	4,003,091
Bonds payable	97,200,000	97,200,000
Total liabilities	151,725,503	121,201,769
Net assets:		
Unrestricted	126,126,561	219,853,852
Temporarily restricted	179,988,153	119,623,060
Permanently restricted	166,762,395	183,540,673
Total net assets	472,877,109	523,017,585
Total liabilities and net assets	$ 624,602,612	644,219,354

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2008
(with summarized financial information for the year ended December 31, 2007)

	Unrestricted	Temporarily Restricted	Permanently Restricted	2008 Total	2007 Total
Revenue and other support:					
Public support—contributions and non-Federal grant awards	$ 18,753,007	71,018,809	12,906,256	102,678,072	71,633,188
Federal grant awards	27,728,155	–	–	27,728,155	30,887,232
Indirect cost allowances	22,079,594	–	–	22,079,594	19,634,082
Investment return utilized	16,956,184	–	–	16,956,184	18,299,207
Program fees	4,634,167	–	–	4,634,167	4,103,754
Publications sales	10,998,768	–	–	10,998,768	10,404,661
Dining services	4,074,984	–	–	4,074,984	3,640,595
Rooms and apartments	3,088,435	–	–	3,088,435	2,804,675
Royalty and licensing fees	1,250,028	–	–	1,250,028	1,092,311
Miscellaneous	1,049,986	–	–	1,049,986	910,490
Net assets released from restrictions	30,575,759	(30,575,759)	–	–	–
Total revenue and other support	141,189,067	40,443,050	12,906,256	194,538,373	163,410,195
Expenses:					
Research	69,062,315	–	–	69,062,315	65,315,923
Educational programs	15,259,681	–	–	15,259,681	12,017,459
Publications	10,764,090	–	–	10,764,090	10,241,091
Banbury Center conferences	1,309,337	–	–	1,309,337	1,342,587
Dolan DNA Learning Center programs	1,476,222	–	–	1,476,222	1,413,157
Watson School of Biological Sciences programs	3,138,774	–	–	3,138,774	3,206,295
General and administrative	14,545,349	–	–	14,545,349	13,111,199
Dining services	5,345,228	–	–	5,345,228	5,109,292
Total expenses	120,900,996	–	–	120,900,996	111,757,003
Excess of revenue and other support over expenses	20,288,071	40,443,050	12,906,256	73,637,377	51,653,192
Other changes in net assets:					
Investment (loss)/return including amount utilized	(59,296,552)	(4,308,727)	(29,684,534)	(93,289,813)	8,259,753
Change in fair value of interest-rate swap	(29,526,602)	–	–	(29,526,602)	(3,076,548)
Write-off of terminated bond insurance	(961,438)	–	–	(961,438)	–
Change in net asset classification based on change in law	(24,230,770)	24,230,770	–	–	–
(Decrease) increase in net assets	(93,727,291)	60,365,093	(16,778,278)	(50,140,476)	56,836,397
Net assets at beginning of year	219,853,852	119,623,060	183,540,673	523,017,585	466,181,188
Net assets at end of year	$ 126,126,561	179,988,153	166,762,395	472,877,109	523,017,585

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 2008
(with comparative financial information for the year ended December 31, 2007)

	2008	2007
Cash flows from operating activities:		
(Decrease) increase in net assets	$ (50,140,476)	56,836,397
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Change in fair value of interest-rate swap	29,526,602	3,076,548
Depreciation and amortization	7,056,947	6,397,045
Net depreciation (appreciation) in fair value of investments	83,339,569	(17,332,229)
Contributions restricted for long-term investment	(28,230,901)	(9,760,257)
Changes in assets and liabilities:		
Decrease in accounts receivable	277,753	325,682
Decrease (increase) in grants receivable	439,731	(1,177,750)
Increase in contributions receivable	(30,956,223)	(26,406,799)
Decrease (increase) in publications inventory	107,133	(201,657)
Increase in prepaid expenses and other assets	(9,961)	(329,586)
Decrease (increase) in restricted use assets	423,656	(392,593)
Increase (decrease) in accounts payable and accrued expenses	4,152,607	(560,202)
(Decrease) increase in deferred revenue	(41,091)	417,178
Net cash provided by operating activities	15,945,346	10,891,777
Cash flows from investing activities:		
Capital expenditures	(46,536,698)	(34,659,089)
Proceeds from sales and maturities of investments	159,193,212	84,438,846
Purchases of investments	(122,321,888)	(92,610,279)
Net change in investment in employee residences	1,208,853	88,191
Net cash used in investing activities	(8,456,521)	(42,742,331)
Cash flows from financing activities:		
Permanently restricted contributions	12,906,256	6,786,787
Contributions restricted for investment in land, buildings and equipment	15,324,645	2,973,470
(Increase) decrease in contributions receivable	(6,632,434)	2,540,317
Decrease in deposits with bond trustee	17,596,724	16,531,481
(Decrease) increase in accounts payable relating to capital expenditures	(3,114,384)	5,069,642
Deposits with swap counterparty	(17,380,247)	–
Payment of conversion costs on bonds payable	(500,701)	–
Write-off of terminated bond insurance	961,438	–
Repayment of notes payable	–	(38,087)
Net cash provided by financing activities	19,161,297	33,863,610
Net increase in cash and cash equivalents	26,650,123	2,013,056
Cash and cash equivalents at beginning of year	25,576,470	23,563,414
Cash and cash equivalents at end of year	$ 52,226,593	25,576,470
Supplemental disclosures: Interest paid	$ 4,153,373	3,812,914

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 2008.

GRANTS January 1–December 31, 2008

COLD SPRING HARBOR LABORATORY

Grantor	Program/Principal Investigator	Duration of Grant		2008 Funding†	
FEDERAL GRANTS					
NATIONAL INSTITUTES OF HEALTH					
Program Project Support	Drs. Hannon/Krainer/Lowe/ Stillman/Tansey	01/01/07	12/31/11	$ 4,539,728	
	Dr. Stillman/CSHL Cancer Center Core	08/15/05	07/31/10	4,126,879	
	Drs. Lowe/Hannon/S. Muthuswamy	04/01/04	03/31/09	913,567	
	Dr. Huang	09/08/06	08/31/11	699,657	
Merit Award Support	Dr. Tonks	08/01/91	03/31/09	668,025	
Research Support	Dr. Dubnau	09/15/04	06/30/09	346,786	
	Dr. Enikolopov	07/01/07	06/30/09	189,000	
	Dr. Hannon	09/01/05	08/31/09	330,161	
	Dr. Huang	07/15/05	04/30/09	371,658	
	Dr. Joshua-Tor	07/01/07	06/30/12	412,020	
	Dr. Joshua-Tor	07/01/05	06/30/09	282,059	
	Dr. Joshua-Tor	02/01/06	01/31/10	308,597	
	Dr. Koulakov	02/01/08	01/31/12	420,000	*
	Dr. Krainer	09/21/07	08/31/11	643,281	
	Dr. Lowe	07/01/99	07/31/09	409,452	
	Dr. Martienssen	08/01/07	07/31/11	412,000	
	Dr. Mills	12/26/07	11/30/12	347,331	
	Dr. Mitra	03/04/05	02/28/09	474,637	
	Dr. Mitra	02/01/08	12/31/09	226,800	*
	Dr. Mittal	04/01/04	06/30/08	148,107	
	Dr. S. Muthuswamy	03/01/03	08/31/09	240,000	
	Drs. Powers/Lowe	12/02/06	11/30/11	463,709	
	Dr. Sebat	04/01/07	03/31/10	711,784	
	Dr. Sebat	09/30/05	07/31/10	404,440	
	Dr. Skowronski	05/01/08	04/30/13	420,000	*
	Dr. D. Spector	04/01/08	03/31/11	642,308	*
	Dr. Stein	05/04/07	05/31/08	204,297	
	Dr. Stein	09/25/06	06/30/09	400,072	
	Dr. Stein	04/01/07	02/28/11	965,953	
	Dr. Stenlund	02/01/08	01/31/12	244,999	*
	Dr. Stillman	06/01/08	05/31/12	602,420	*
	Dr. Svoboda	03/01/04	02/28/09	325,452	
	Dr. Svoboda	12/04/98	11/20/09	59,466	
	Dr. Tansey	05/01/07	04/30/11	378,049	
	Dr. Tonks	07/01/05	06/30/09	369,649	
	Dr. Tonks	07/21/06	05/31/10	289,553	

* New grants awarded in 2008
† Includes direct and indirect costs

Grantor	Program/Principal Investigator	Duration of Grant		2008 Funding†
	Dr. Van Aelst	07/01/08	03/31/13	378,000 *
	Dr. Van Aelst	05/01/03	04/30/09	42,000
	Dr. Zador	09/18/08	07/31/13	357,000 *
	Dr. Zhang	05/01/08	02/28/11	700,000 *
	Dr. Zhang	08/13/07	07/31/11	319,200
Research Subcontracts				
NIH/Affymetrix, Inc. Consortium Agreement	Dr. Gingeras	07/01/08	12/31/08	89,694 *
NIH/Affymetrix, Inc. Consortium Agreement	Dr. Hannon	09/27/07	12/31/08	538,398
NIH/Affymetrix, Inc. Consortium Agreement	Dr. Gingeras	07/01/08	12/31/08	159,029 *
NIH/Brookhaven National Laboratory Consortium Agreement	Dr. Stillman	09/01/06	08/31/11	37,724
NIH/Columbia University Consortium Agreement	Dr. Lowe	08/18/06	07/31/11	396,086
NIH/Columbia University Consortium Agreement	Dr. Wigler	09/01/06	05/31/10	286,067
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	09/29/11	252,000
NIH/Ludwig Institute for Cancer Research Consortium Agreement	Dr. Zhang	09/29/08	06/30/13	227,641 *
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Hannon	05/04/07	06/05/08	21,123
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Mittal	04/01/04	03/31/09	34,718
NIH/Oxford University Consortium Agreement	Dr. Stein	03/01/06	02/28/11	155,862
NIH/Rutgers University Consortium Agreement	Dr. Mitra	12/08/04	11/30/09	147,668
NIH/University of Colorado Consortium Agreement	Dr. Hannon	06/15/08	05/31/10	50,400 *
Fellowship Support	Dr. Aravin	05/01/08	04/30/09	97,200 *
	Dr. Chicas	04/01/06	03/31/09	53,992
	Dr. Demas	02/16/07	02/15/10	23,475
	Dr. Slotkin	09/15/06	09/14/09	48,796
	Dr. Yang	03/01/06	02/28/09	53,992
Graduate Training Support	Dr. Joshua-Tor, Watson School of Biological Sciences	07/01/02	06/30/12	205,229
Course Support	Advanced Techniques in Molecular Neuroscience	07/01/01	06/30/12	88,001
	C. elegans	08/01/98	08/31/11	78,345
	Cell and Developmental Biology of *Xenopus*	04/01/93	03/31/09	21,880
	Cellular Biology of Addiction	07/01/05	06/30/09	59,087
	Computational and Comparative Genomics	06/06/91	07/31/13	47,397
	Eukaryotic Gene Expression	01/01/83	03/31/11	114,832
	Imaging Structure and Function in the Nervous System	07/01/01	06/30/12	74,656
	Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging	07/01/98	08/31/10	64,547
	Integrated Data Analysis for High-throughput Biology	09/01/07	04/30/12	73,941

* New grants awarded in 2008
† Includes direct and indirect costs

Grantor	Program/Principal Investigator	Duration of Grant		2008 Funding†	
	Molecular Embryology of the Mouse	01/01/83	03/31/11	93,481	
	Neurobiology of *Drosophila*	07/01/01	06/30/12	60,605	
	Programming for Biology	07/01/07	08/31/09	56,823	
	Protein Purification and Characterization	01/01/83	03/31/11	80,564	
	Proteomics	07/01/03	06/30/11	76,096	
	X-ray Methods in Structural Biology	07/01/07	06/30/12	72,051	
	Yeast Genetics and Genomics	07/01/07	05/31/10	48,925	
Meeting Support	Axon Guidance, Synaptogenesis, and Neural Plasticity	04/01/08	03/31/09	15,000	*
	The Biology of Genomes	04/01/08	03/31/13	36,000	*
	The Cell Cycle	03/01/06	02/28/11	4,000	
	Gene Expression and Signaling in the Immune System	04/01/08	03/31/13	10,000	*
	Germ Cells	07/01/08	06/30/13	6,000	*
	Mechanisms and Models of Cancer	08/01/08	07/31/13	6,000	*
	Molecular Chaperones and Stress Responses	04/01/04	03/31/09	13,000	
	Molecular Genetics of Aging	03/01/08	02/28/13	29,155	*
	Mouse Molecular Genetics	09/01/08	08/31/09	6,000	*
	Pharmacogenomics	08/01/04	07/31/09	21,029	
	PTEN Pathways and Targets	03/01/06	02/28/11	7,000	
	73rd Symposium: Control and Regulation of Stem Cells	05/01/08	04/30/09	11,500	*
NATIONAL SCIENCE FOUNDATION					
Multiple Project Award Support	Dr. Hannon	09/01/06	08/31/08	400,000	
	Dr. Jackson	07/01/05	06/30/10	1,033,001	
	Dr. Ware	10/01/07	09/30/11	1,927,925	
Research Support	Dr. Jackson	04/01/07	03/31/10	145,000	
	Dr. Timmermans	09/01/06	08/31/09	140,000	
	Dr. Timmermans	09/01/06	08/31/09	156,874	
	Dr. Ware	06/15/08	05/31/11	262,382	*
	Dr. Zador	10/01/07	09/30/09	10,750	
Research Subcontracts					
NSF/Cornell University Consortium Agreement	Dr. Timmermans	09/01/08	08/31/12	514,843	*
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	10/01/04	09/30/09	258,759	
NSF/North Carolina State University Consortium Agreement	Drs. Martienssen/Vaughn	10/01/04	09/30/09	370,594	
NSF/University of Arizona Consortium Agreement	Drs. Stein/Ware/Martienssen/Micklos/Vaughn	02/01/08	01/31/13	1,107,559	*
NSF/University of California, Berkeley, Consortium Agreement	Dr. Jackson	08/01/06	07/31/11	215,394	
NSF/University of California, Davis, Consortium Agreement	Dr. Martienssen	09/01/06	08/31/10	263,712	
NSF/University of California, Davis, Consortium Agreement*	Dr. Ware	09/01/08	08/31/11	103,570	*
NSF/University of Florida Consortium Agreement	Dr. Martienssen	08/01/07	07/31/09	102,010	
NSF/University of Wisconsin Consortium Agreement	Drs. Stein/Ware	01/01/04	12/31/08	220,892	
NSF/Washington University Consortium Agreement	Drs. McCombie/Martienssen/Stein/Ware	11/15/05	10/31/09	782,667	

* New grants awarded in 20088
† Includes direct and indirect costs

Grantor	Program/Principal Investigator	Duration of Grant		2008 Funding†	
Fellowship Support	C. Malone	06/01/07	05/31/09	40,500	
	Dr. Eveland	10/01/08	09/30/10	60,000	*
Undergraduate Training Support	Dr. Joshua-Tor, Watson School of Biological Sciences	04/15/05	03/31/10	60,000	
Course Support	Advanced Bacterial Genetics	07/15/04	06/30/09	79,398	
	Cell and Developmental Biology of *Xenopus*	05/01/07	04/30/10	20,000	
	Computational Cell Biology	04/15/08	03/31/11	92,165	*
	Molecular Techniques in Plant Science	07/01/07	06/30/10	44,475	
	Workshop on Cereal Genomics	11/01/08	10/31/09	47,384	*
Meeting Support	Molecular Genetics of Bacteria and Phages	04/15/08	03/31/09	5,000	*
	The Plant Epigenome: Frontiers in Gene Regulation	03/15/08	02/28/09	30,000	*
UNITED STATES DEPARTMENT OF AGRICULTURE					
Research Support	Dr. Jackson	09/01/08	08/31/11	136,195	*
	Dr. McCombie	09/15/04	09/14/09	222,611	
	Dr. Stein	09/22/03	09/15/09	22,000	
	Dr. Timmermans	09/15/06	09/14/09	137,166	
UNITED STATES DEPARTMENT OF THE ARMY					
Research Support	Dr. Hannon	02/01/06	01/31/09	169,500	
	Dr. Hannon	02/15/06	02/14/09	169,500	
	Dr. Hannon	09/01/08	08/31/13	608,696	*
	Dr. Lazebnik	12/01/06	11/30/09	209,999	
	Drs. Lucito/Tonks	08/01/08	07/31/09	504,000	*
	Dr. S. Muthuswamy	06/01/08	05/31/13	642,996	*
	Dr. Powers	07/01/07	06/30/10	168,000	
Research Subcontracts					
U.S. Army/New York University School of Medicine Consortium Agreement	Dr. Lucito	09/27/04	09/26/09	31,673	
Fellowship Support	Dr. He	12/15/08	12/14/11	32,400	*
	D. Khalil	12/01/06	11/30/09	33,300	
	D. Simpson	09/01/06	08/31/09	32,400	
MISCELLANEOUS SOURCES OF FUNDING					
Equipment Support					
Mrs. Kathryn W. Davis	Dr. Hannon	01/01/08	12/31/08	400,000	*
F.M. Kirby Foundation, Inc.	Dr. Osten	12/01/08	11/30/09	100,000	*
Program Project Support					
The Leukemia & Lymphoma Society of America	Dr. Lowe	10/01/08	09/30/13	1,250,000	*
Pioneer Hi-Bred International, Inc.	Drs. Jackson/Lukowitz/Martienssen/Timmermans/Ware	07/01/07	06/30/12	1,600,000	
The Simons Foundation/Autism	Dr. Wigler	07/01/08	06/30/11	6,175,430	*

* New grants awarded in 2008
† Includes direct and indirect costs

Grantor	Program/Principal Investigator	Duration of Grant		2008 Funding†	
The Simons Foundation/Center for Quantitative Biology	Dr. Wigler	09/01/08	08/31/11	1,500,000	*
Theodore R. and Vada S. Stanley	Drs. Watson/McCombie/Sebat	07/01/07	06/30/12	5,000,000	
Research Support					
American Cancer Society	Dr. Mills	07/01/06	06/30/10	240,000	
American Cancer Society	Dr. Wigler	01/01/06	12/31/10	60,000	
American Cancer Society	Dr. Wigler	01/01/05	12/31/08	10,000	
Anonymous Gift	Dr. Zador	09/01/07	08/31/09	500,000	
Anonymous Gift	Dr. Sebat	09/01/07	08/31/09	251,174	
Anonymous Gift	Dr. Sebat	04/01/08	03/31/09	1,000	*
Autism Speaks, Inc.	Dr. Zador	07/01/07	06/30/10	150,000	
Mr. Donald E. Axinn	Drs. Lowe/Hannon	01/01/08	12/31/08	250,000	*
Geoffrey Beene Cancer Center/Sloan-Kettering Institute for Cancer Research	Dr. Hicks	08/01/08	07/31/10	45,260	*
The Breast Cancer Research Foundation	Dr. Wigler	10/01/08	09/30/09	250,000	*
The Breast Cancer Research Foundation	Dr. Wigler	01/01/08	12/31/08	225,000	*
Coleman Fung Foundation Inc.	Dr. Zador	02/01/07	01/31/10	50,000	
DART Neurogenomics Project	Drs. Cline/Dubnau/Tully/Zhong	10/01/04	12/31/08	618,326	
The Shelby Cullom Davis Foundation	Dr. Hannon	01/15/07	01/14/11	1,000,000	
Find a Cure Today (F.A.C.T.)	Dr. Egeblad	12/01/08	11/30/09	30,000	*
Families of Spinal Muscular Atrophy	Dr. Krainer	03/01/07	02/28/09	98,532	
The Joni Gladowsky Breast Cancer Foundation	Dr. Egeblad	12/01/08	11/30/09	40,000	*
Irving A. Hansen Memorial Foundation	Dr. Tansey	08/01/08	07/31/09	20,000	*
The Thomas Hartman Foundation	Dr. Tonks	07/01/07	06/30/09	179,418	
Jo-Ellen and Ira Hazan	Dr. Enikolopov	12/01/08	11/30/09	200,000	*
Hearts for Cancer	Dr. S. Muthuswamy	12/01/07	11/30/09	3,535	
Human Frontier Science Program	Dr. Dubnau	10/01/06	09/30/09	125,000	
Isis Pharmaceuticals, Inc.	Dr. Krainer	11/01/08	10/31/09	125,000	*
Joan's Legacy: The Joan Scarangello Foundation to Conquer Lung Cancer	Dr. Sordella	12/01/07	11/30/09	50,000	
Long Islanders Against Breast Cancer (LIABC)	Dr. Wigler	2008		2,000	*
Roni Kamin-McGuffog and Charles McGuffog	Dr. Furukawa	01/01/08	12/31/08	1,000	*
V. Kann Rasmussen Foundation	Dr. Trotman	09/01/07	08/31/12	50,000	
The Karches Foundation	Dr. Wigler	07/01/05	06/30/10	480,131	
W.M. Keck Foundation	Dr. Mitra	07/01/06	06/30/09	500,000	
The Charles H. Leach, II Foundation	Dr. Enikolopov	06/01/08	05/31/09	5,000	*
The Long Island 2-Day Walk to Fight Breast Cancer	Dr. Egeblad	11/01/08	10/31/09	31,500	*
Manhasset Women's Coalition Against Breast Cancer	Dr. Egeblad	11/01/08	10/31/09	12,000	*
Carol Marcincuk Fund	Dr. Lucito	01/01/08	12/31/08	6,075	*
The G. Harold and Leila Y. Mathers Charitable Foundation	Dr. Huang	07/01/07	06/30/10	199,916	
The Elizabeth McFarland Breast Cancer Fund	Dr. Wigler	12/01/08	11/30/09	52,533	*
The Don Monti Memorial Research Foundation	Dr. Lowe	03/01/08	02/28/09	500,000	*
Louis Morin Charitable Trust	Drs. Kepecs/Zador	12/01/08	11/30/09	70,000	*
Muscular Dystrophy Association, Inc.	Dr. Krainer	07/01/07	06/30/10	254,000	
New York State Foundation for Science, Technology and Innovation (NYSTAR)	Dr. Paddison	03/01/07	02/28/09	76,733	

* New grants awarded in 2008
† Includes direct and indirect costs

Grantor	Program/Principal Investigator	Duration of Grant		2008 Funding[†]	
New York State Stem Cell Science (NYSTEM)	Drs. Enikolopov/Timmermans/Van Aelst	04/01/08	03/31/09	380,933	*
The Pierre and Pamela Omidyar Fund	Dr. Powers	01/01/08	12/31/08	250,000	*
Philips Research North America	Drs. Wigler/Lucito	04/01/07	03/31/09	603,178	
Christina Renna Foundation, Inc.	Dr. Van Aelst	01/01/08	12/31/08	25,000	*
Diane Emdin Sachs Memorial Fund	Dr. Tansey	05/01/08	04/30/09	26,630	*
The Simons Foundation	Dr. Huang	10/01/07	09/30/10	440,805	
The Simons Foundation	Dr. Mills	01/01/07	12/31/09	527,437	
Mary F. Smith Family Foundation	Dr. Egeblad	10/01/08	09/30/09	1,000	*
Starr Cancer Consortium	Dr. Hannon	07/01/08	06/30/10	524,071	*
Starr Cancer Consortium	Dr. Krainer	11/01/07	10/31/09	300,000	
Starr Cancer Consortium	Dr. Lowe	07/01/08	06/30/10	60,000	*
Starr Cancer Consortium	Drs. Lowe/Hannon	11/01/07	10/31/09	480,000	
Starr Cancer Consortium	Dr. McCombie	07/01/08	06/30/09	300,000	*
Starr Cancer Consortium	Drs. Powers/Lucito	11/01/07	10/31/09	378,343	
Starr Cancer Consortium	Dr. Van Aelst	11/01/07	10/31/09	240,000	
Starr Cancer Consortium	Dr. Wigler	11/01/07	10/31/09	120,000	
Starr Cancer Consortium	Dr. Powers	11/01/07	10/31/09	300,000	
Swim Across America	Dr. Sordella	11/01/08	10/31/09	50,000	*
The V Foundation	Dr. Powers	11/01/08	10/31/11	200,000	*
The V Foundation	Dr. Trotman	11/01/08	10/31/10	50,000	*
Waldbaum's Foundation	Dr. Egeblad	09/01/08	08/31/09	10,000	*
West Islip Breast Cancer Coalition for Long Island Inc.	Dr. Egeblad	11/01/08	10/31/09	10,000	*
Whitehall Foundation, Inc.	Dr. Kepecs	06/01/08	05/31/11	75,000	*
Women in Science	Dr. D. Spector	01/01/08	12/31/08	61,800	*
Women's Insurance Network of Long Island	Dr. S. Muthuswamy	06/01/08	05/31/09	16,900	*
Fellowship Support					
The Rita Allen Foundation	Dr. Trotman	09/01/07	08/31/10	100,000	
American Association for Cancer Research	W. Xue	09/01/08	08/31/11	30,000	*
American Cancer Society	Dr. Karginov	01/01/07	12/31/09	46,000	
Autism Speaks, Inc.	Dr. Xiong	10/01/08	09/30/10	44,000	*
The Binational Agricultural Research and Development Fund	Dr. A. Goldshmidt	10/10/08	10/19/09	37,000	*
Arnold and Mabel Beckman Foundation	Watson School of Biological Sciences	09/01/05	08/31/10	350,000	
Terri Brodeur Breast Cancer Foundation	Dr. Chatterjee	01/01/07	12/31/08	50,000	
Cashin Family Fund	Watson School of Biological Sciences	09/01/08	08/31/09	40,000	*
The Mary K. Chapman Foundation	Dr. Kuhn	04/01/08	06/30/08	18,730	*
The Mary K. Chapman Foundation	Dr. Xu	07/01/08	03/31/09	66,270	*
The Jane Coffin Childs Memorial Fund for Medical Research	Dr. Kuhn	07/01/08	06/30/11	45,500	*
CSHL Association Fellowship	New Investigator Support	2008		280,000	*
The Danish Cancer Society	Dr. Jensen	01/01/07	12/31/08	70,000	
Michel David-Weill	Dr. Albeanu	09/01/06	08/31/09	200,000	
Ellison Medical Foundation	Dr. Chien	07/01/07	06/30/09	49,250	
Engelhorn Scholarship Program	Watson School of Biological Sciences	09/01/00	12/31/08	49,980	
European Molecular Biology Organization	Dr. Demir	01/01/08	06/30/08	16,591	*
Fondation pour la Recherche Medicale	Dr. Anczukow-Camarda	01/01/08	12/31/08	32,744	*
DFG (German Research Foundation)	Dr. Zuber	02/01/06	01/31/09	3,600	
The Allen and Lola Goldring Foundation	Dr. Stillman	09/01/08	08/31/09	75,000	*
The Lita Annenberg Hazen Foundation	Watson School of Biological Sciences	05/01/08	04/30/18	10,000	*
The Heart and Stroke Foundation	Dr. Boivin	07/01/06	06/30/08	19,558	
Human Frontier Science Program	Dr. Kvitsiani	09/01/08	08/31/11	46,400	*

* New grants awarded in 2008
[†] Includes direct and indirect costs

Grantor	Program/Principal Investigator	Duration of Grant		2008 Funding†
Human Frontier Science Program	Dr. Nadif Kasri	09/01/06	11/30/09	45,500
Human Frontier Science Program	Dr. Schalch	08/01/07	08/01/10	50,220
The Japan Society for the Promotion of Science (JSPS)	Dr. Kawakami	04/01/08	03/31/10	39,000 *
Annette Kade Charitable Trust	Watson School of Biological Sciences	03/01/07	03/31/09	50,000
The Karp Foundation, Inc.	Dr. Karakas	10/16/08	10/15/09	50,000 *
The Leukemia & Lymphoma Society	Dr. Krizhanovsky	07/01/06	06/30/09	50,000
The Leukemia & Lymphoma Society	Dr. Semighini	07/01/08	06/30/11	55,000 *
Merck Research Laboratories	Watson School of Biological Sciences	01/01/08	12/31/08	25,000 *
NARSAD	Dr. Encinas	07/01/07	06/30/09	30,000
NARSAD	Dr. Park	07/01/08	06/30/10	30,000 *
The Robert Leet and Clara Guthrie Patterson Trust	Dr. Demir	07/01/08	06/30/10	44,500 *
The Robert Leet and Clara Guthrie Patterson Trust	Dr. Kvitsiani	01/15/08	08/31/08	27,813 *
The Robert Leet and Clara Guthrie Patterson Trust	Dr. Lima	01/15/07	01/14/09	10,704
The Robert Leet and Clara Guthrie Patterson Trust	Dr. Oviedo	01/15/08	01/14/10	50,000 *
Spanish Ministry of Education and Science	Dr. Fernandez-Marco	09/01/07	08/31/09	38,248
Lauri Strauss Leukemia Foundation	Dr. Semighini	03/01/08	02/28/09	15,000 *
The Swartz Foundation	Dr. Zador	01/01/08	12/31/08	333,333 *
The Swartz Foundation	Drs. Koulakov/Tsigankov	01/01/08	12/31/08	55,000 *
The Swartz Foundation	Drs. Mainen/Felsen	01/01/08	12/31/08	55,000 *
The Swartz Foundation	Drs. Zador/Jaramillo	01/01/08	12/31/08	55,000 *
The Swartz Foundation	Drs. Zador/Oswald	01/01/08	12/31/08	55,000 *
The Swartz Foundation	Dr. Zador	01/01/08	12/31/08	15,000 *
Training Support				
Cornelius N. Bliss Memorial Fund	Undergraduate Research Program	2008		2,700 *
Hunter College	Undergraduate Research Program	2008		7,000 *
Steamboat Foundation	Undergraduate Research Program	05/01/08	04/30/09	12,000 *
William Townsend Porter Foundation	Undergraduate Research Program	04/01/08	03/31/09	10,000 *
Course Support				
American Brain Tumor Association	Mechanisms of Neural Differentiation and Brain Tumors	04/01/08	03/31/09	45,000 *
The American Society for Cell Biology	ASCB Minority Affairs Committee Grant	02/01/08	01/31/09	15,000 *
Applied Biosystems	Revolutionary Sequencing Technologies and Applications	06/01/08	05/31/09	26,000 *
Autism Speaks, Inc.	Workshop on Biology of Social Cognition	05/01/07	06/01/10	15,000
Howard Hughes Medical Institute	Neurobiology Course Support	01/01/91	12/31/11	750,000
IBRO–Society for Neuroscience	Student Support–Course Scholarships	01/05/06	03/31/09	20,775
Illumina, Inc.	Revolutionary Sequencing Technologies and Applications	06/01/08	05/31/09	26,000 *
Nancy Lurie Marks Family Foundation	Workshop on Biology of Social Cognition	06/01/07	06/01/10	15,000
Novartis Institutes for BioMedical Research, Inc.	Proteomics Course Support	06/01/06	09/30/08	129,859
Roche Diagnostics Corporation	Revolutionary Sequencing Technologies and Applications	06/01/08	05/31/09	26,000 *
The Simons Foundation	Workshop on Biology of Social Cognition	06/01/07	06/01/10	15,000
Meeting Support				
Abbott Laboratories	Gene Expression and Signaling in the Immune System	01/01/08	12/31/08	5,000 *

* New grants awarded in 2008
† Includes direct and indirect costs

Grantor	Program/Principal Investigator	Duration of Grant		2008 Funding[†]	
California Institute of Technology	Engineering Principles for Biological Systems	12/01/08	11/30/09	20,000	*
Champalimaud Foundation	73rd Symposium: Control and Regulation of Stem Cells	03/01/08	02/28/09	175,000	*
Chemocentryx	Gene Expression and Signaling in the Immune System	01/01/08	12/31/08	2,500	*
Elan Pharmaceuticals, Inc.	Gene Expression and Signaling in the Immune System	01/01/08	12/31/08	4,000	*
Genentech	Gene Expression and Signaling in the Immune System	01/01/08	12/31/08	3,000	*
Hudson-Alpha Institute for Biotechnology	The Biology of Genomes	03/01/07	02/29/09	24,000	
IRX Therapeutics, Inc.	Gene Expression and Signaling in the Immune System	01/01/08	12/31/08	1,500	*
The Lalor Foundation	Conference on Germ Cells–Support for Young Investigators	01/01/08	12/31/08	5,000	*
The Lehrman Institute	Human Genome	09/01/07	08/31/08	25,000	
March of Dimes Foundation	Conference on Germ Cells	08/01/08	07/31/09	3,500	*
Merck Research Laboratories	Neurodegenerative Diseases: Biology and Therapeutics	06/01/08	05/31/09	2,000	*
Merck Research Laboratories	Molecular Genetics of Bacteria and Phages	04/01/08	03/31/09	2,500	*
Merck Research Laboratories	Axon Guidance, Synaptogenesis, and Neural Plasticity	04/01/08	03/31/09	2,000	*
Merck Research Laboratories	Mechanisms and Models of Cancer	01/01/08	12/31/08	25,000	*
New York State Stem Cell Science (NYSTEM)	Stem Cell Training Program	11/01/08	10/31/09	118,920	*
OSI Pharmaceuticals	Epithelial–Mesenchymal Transition	03/01/08	02/28/09	48,265	*
OSI Pharmaceuticals	PTEN Pathways	03/01/08	02/28/09	16,735	*
Research and Diagnostic Systems, Inc.	Gene Expression and Signaling in the Immune System	01/01/08	12/31/08	2,500	*
Roche Diagnostics Corporation	Personal Genomes Conference	10/01/08	09/30/09	25,000	*
Roche Diagnostics Corporation	The Biology of Genomes	03/01/07	02/29/09	40,000	
Schering-Plough	Gene Expression and Signaling in the Immune System	01/01/08	12/31/08	2,500	*
Vertex Pharmaceuticals Incorporated	Gene Expression and Signaling in the Immune System	01/01/08	12/31/08	2,000	*
Wyeth Pharmaceuticals	Harnessing Immunity to Prevent and Treat Disease	12/01/08	11/30/09	10,000	*
Library Support					
New York State		07/01/07	06/30/09	8,699	

* New grants awarded in 2008
[†] Includes direct and indirect costs

DOLAN DNA LEARNING CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2008 Funding*[†]
FEDERAL GRANTS			
National Institutes of Health	Science Education Partnership Award (SEPA): Nationwide Dissemination of *Inside Cancer* Internet Site	08/07–07/09	$ 140,653
National Science Foundation	Course, Curriculum, and Laboratory Instruction (CCLI) Program: Nationwide Dissemination of RNAi Curriculum	09/07–8/09	132,740
National Science Foundation	Plant Genome Initiative Educational Outreach: Construction and Nationwide Dissemination of *Dynamic Gene* Internet Site	12/05-11/08	3,939
AAAS/NSF	National Science Digital Library: Meta-tagging DNALC Internet Content for BiosciEdNet	10/05–09/09	71,469
Washington University/NSF	Plant Genome Initiative Educational Outreach: Multimedia Materials on Maize Genome Sequencing	11/05–10/08	15,611
Cornell University/NSF	Plant Genome Initiative Educational Outreach: Minority Fellows and Regional Plant Genomics Footlockers	09/08–08/09	13,114
University of Arizona/NSF	*iPlant* Collaborative: Cyberinfrastructure for the Biological Sciences	02/08–01/09	237,904
NONFEDERAL GRANTS			
Amgen Foundation	Amgen Leadership Symposium	04/05–12/08	$ 106,469
Dana Foundation	*Genes to Cognition* (*G2C*) *Online* Internet Site Development	10/04–12/08	265,552
Dialog Gentechnik	DNALC Licensing	2008	24,975
Hewlett Foundation	*Genes to Cognition* (*G2C*) *Online* Internet Site Evaluation	10/05–10/09	86,702
HHMI Foundation	Pre-College Science Education Initiative: NYC Teacher Training	09/07–08/09	136,971
New York State Education Dept.	Excelsior Scholars Program for 7th Grade Math & Science Students	05/08–10/08	42,794
North Shore–LIJ Health System	DNALC *West* Operating Support	2008	50,000
Porter Foundation	Scholarships for Minority and Disadvantaged Students	03/08–03/10	425

The following schools each contributed $1000 or more for participation in the *Curriculum Study* Program:

School	Amount
Bellmore–Merrick Central High School District	$1,250
Bethpage Union Free School District	1,500
Commack Union Free School District	3,000
East Meadow Union Free School District	1,500
Elwood Union Free School District	1,500
Fordham Preparatory School	1,500
Garden City Union Free School District	1,500
Great Neck Union Free School District	1,500
Half Hollow Hills Central School District	3,000
Harborfields Central School District	1,500
Herricks Union Free School District	3,000
Huntington Union Free School District	1,500
Island Trees Union Free School District	1,500
Jericho Union Free School District	1,500
Locust Valley Central School District	$1,500
Long Beach City School District	1,500
Massapequa Union Free School District	1,500
North Shore Central School District	1,500
Oyster Bay–East Norwich School District	3,000
Plainview–Old Bethpage Central School District	1,500
Port Washington Union Free School District	1,500
Ramaz School	1,500
Sachem Central School District	1,500
South Huntington Union Free School District	1,500
Syosset Central School District	1,500
West Hempstead Union Free School District	1,500
Yeshiva University High School for Girls	1,500

The following schools each contributed $1000 or more for participation in the *Genetics as a Model for Whole Learning* Program:

School	Amount
Amityville Union Free School District	$ 1,200
Bay Shore Union Free School District	5,100
Bellmore Union Free School District	2,000
Bellmore–Merrick Central HS District	17,000
Commack Union Free School District	5,875
Deer Park Union Free School District	1,200
East Meadow Union Free School District	2,550
Elwood Union Free School District	4,200
Floral Park–Bellerose Union Free School District	6,750
Friends Academy, Locust Valley	2,350
Garden City Union Free School District	3,700
Great Neck Union Free School District	16,500
Grover Cleveland Middle School, NJ	1,200
Half Hollow Hills Central School District	7,125
Herricks Union Free School District	2,400
Holy Family Regional School, Commack	1,500
Huntington Union Free School District	6,800
Kings Park Central School District	1,137
Lindenhurst Union Free School District	1,000
Locust Valley Central School District	11,650
Long Beach City School District	1,200
Merrick Union Free School District	2,700
MS 447–The Math & Science Exploratory School, N.Y.	5,000
Mott Hall V, NYC	$ 2,400
North Bellmore Union Free School District	3,300
North Shore Hebrew Academy	1,000
Northport–East Northport Union Free School District	1,600
Oceanside Union Free School District	1,500
Old Westbury School of the Holy Child	3,250
Oratory School of Summit	1,200
Oyster Bay–East Norwich Central School District	2,500
Plainview–Old Bethpage Central School District	3,500
Roberto Clemente Middle School, MD	3,740
Rockville Centre Union Free School District	5,400
Roslyn Union Free School District	3,600
Scarsdale Union Free School District	9,600
St. Dominic Elementary School, Oyster Bay	4,050
St. Edward the Confessor School, Syosset	2,025
St. Isidore School, Riverhead	1,200
St. Joseph School, Garden City	1,000
Syosset Central School District	33,100
Three Village Central School District	6,000
Trinity Lutheran School, Northport	1,000
United Federation of Teachers School, NYC	2,000
Yeshiva Darchei Torah	1,600
Yeshiva of Flatbush	1,100

[†]Includes direct and indirect costs

BANBURY CENTER GRANTS

Grantor	Program	Duration of Grant	2008 Funding
FEDERAL SUPPORT			
NIH–National Institute of Allergy and Infectious Diseases; Office of Rare Diseases	Prion Strains: Origins, Mechanisms, and Implications for Disease	2008	25,000*
NIH–National Institute of Mental Health (through a grant to University of Illinois)	Recent Advance and a Multilevel Analysis from FMRP Biology to Clinical Trials	2008	53,914*
NONFEDERAL SUPPORT			
Meeting Support			
AVI BioPharma, Inc.	Oligonucleotide-directed Splicing: Therapeutic Strategies	2008	10,800*
Clay Mathematics Institute	Algebraic Statistics, Machine Learning, and Lattice Spin Models	2008	13,273*
Cold Spring Harbor–Pioneer Collaborative Research Program	Plant Genetics and Gene Regulation	2008	43,526
Cure Duchenne Foundation	Oligonucleotide-directed Splicing: Therapeutic Strategies	2008	10,800*
The Dana Foundation	How Can We Improve Our Brains?	2008	48,600*
Foundation to Eradicate Duchenne	Oligonucleotide-directed Splicing: Therapeutic Strategies	2008	10,800*
Oliver Grace Professorship Fund	To What Age Should We Work?	2008	43,810*
The W.M. Keck Foundation	The Architectural Logic of Mammalian CNS	2008	33,155
Richard Lounsbery Foundation	Who Are We? Kinship, Ancestry, and Social Identity	2008	46,767*
The Lustgarten Foundation for Pancreatic Cancer Research	Identifying KRAS-targeted Therapeutic Approaches for Pancreatic Cancer	2008	15,181*
Medical Research Council, UK	Prion Strains: Origins, Mechanisms, and Implications for Disease	2008	29,576*
OSI Pharmaceuticals, Inc.	How Will We Be Able to Cure Most Cancers?	2008	56,689*
Prosensa	Oligonucleotide-directed Splicing: Therapeutic Strategies	2008	10,800*
Spinal Muscular Atrophy Foundation	Taking on New Complexities in SMA Biology	2008	54,538*
The Swartz Foundation	Theoretical and Experimental Approaches to Auditory and Visual Attention	2008	51,497*
Stanley Trotman, Jr. Trust	Genes and the Environment: New Strategies for Research on Multiple Sclerosis	2008	22,554*

* New grants awarded in 2008

CORPORATE SPONSOR PROGRAM FOR MEETINGS SUPPORT

The Corporate Sponsor Program continues to provide critical funding for the vigorous meetings program held at Cold Spring Harbor Laboratory, whether at Grace Auditorium on the main Laboratory campus or at the Banbury Center. Without the strong foundation provided by the Program, we could neither plan with confidence for the year's meetings nor introduce new and unusual topics.

We are especially grateful to the companies that joined us in 2008 as the economic difficulties began to take effect. The year 2009 is going to be especially challenging as the number of companies shrinks through takeovers, and companies and foundations adopt austerity measures.

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 8000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, and this is mailed to approximately 30,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory Web site on the Meetings Office and Banbury Center pages. Members in 2008 were:

CORPORATE BENEFACTORS

Amgen Inc.
GlaxoSmithKline
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Novartis Institutes for BioMedical Research

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Epicentre Biotechnologies
Illumina Inc.

DEVELOPMENT

We reached an important milestone in 2008 as our campaign surpassed its $200 million goal. As a result of this campaign, we constructed six new buildings, increasing the Laboratory's research space by more than 40%. This will allow our cancer programs to expand and use novel imaging technologies to determine why and how tumors metastasize. Our neuroscience efforts will grow and increase our understanding of the complex genetic pathways involved in neurological disorders, most notably autism, schizophrenia, and depression. Through significant campaign gifts from the Simons Foundation, the Starr Foundation, and Landon and Lavinia Clay, we launched a new program in Quantitative Biology that will focus on mining genomic data to better understand human health and disease.

Our educational programs benefited from the campaign, which facilitated a much-needed endowment for the DNA Learning Center, allowing for expansion with a new facility in East Harlem that opened in September. The campaign also helped to increase funds for the Watson School of Biological Sciences with the establishment of new graduate school fellowships as well as support for its heralded Undergraduate Research Program.

We would like to thank all of our campaign participants and 2008 donors for their unwavering support and friendship.

Charles V. Prizzi, Vice President for Development

Cold Spring Harbor Laboratory is a nonprofit research and educational institution, chartered by the State of New York. ScienceWatch, an independent rating service, has ranked the Laboratory's molecular biology and genetics programs number one during the last decade. Additionally, *Charity Navigator*, a philanthropic evaluator, has bestowed their highest four-star rating on the Laboratory for seven consecutive years.

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. There are a variety of ways to give to the Laboratory:

Capital and Endowment Campaign Support: CSHL is completing a $200 million capital and endowment campaign to speed the translation of genetic discoveries into diagnostic tests and therapeutics and to expand the Lab's facilities and staff. Endowment gifts can be directed toward supporting cancer, neuroscience, or educational programs. Capital gifts can be made to name laboratories and conference rooms in one of our six new research buildings.

Annual Fund: Donations provide funding for some of the most determined and innovative young researchers in science today and constitute an important investment in innovative research in cancer, neuroscience, plant biology, and bioinformatics.

Science Education: Donations support programs at the DNA Learning Center, where the next generation of scientists learn about genetics in an exciting and interactive environment.

Planned and Estate Gifts: Individuals who inform us of their intention to make a gift to CSHL from their estate are invited to become members of The Harbor Society. Estate gifts help to ensure that CSHL will continue to pursue its mission for many years to come.

For additional information, please contact the Vice President for Development, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6865

President's Council

The President's Council is comprised of distinguished individuals who gather each spring and fall for stimulating discussions on scientific topics of broad public interest that are related to CSHL research. Members of the Council contribute at least $25,000 annually to support CSHL Fellows, exceptionally talented young scientists who show unusual potential early in their careers.

In 2008, the Council's meetings focused on plant biology to commemorate the 100th anniversary of George Shull's demonstration at CSHL of hybrid vigor in corn, which ushered in the U.S. agricultural revolution. Today, CSHL plant biologists are helping to develop technologies for improving crop yield, enhance nutritional value of food crops, and speed the development of viable biofuels.

Our April meeting was hosted by Trustee Andrew Solomon and John Habich Solomon at their home in New York City. The gathering featured a lecture and lab demonstration on genetically modified foods. In October, more than 60 people gathered for a two-day meeting on biofuels. They heard in-depth talks from leaders in industry, policy, and research on fuel consumption and energy production, which focused on initiatives for making low-carbon biofuels an economic reality. The meeting spanned three CSHL campuses, beginning at the Banbury Conference Center with a talk by the former president of Shell Oil, John Hofmeister, who focuses today on challenging industry, government, and grassroots organizations to create an alternative energy plan for the U.S, and ending with a tour of CSHL's farm and greenhouses and a lively talk on the history of plant research at the Laboratory.

President

Dr. Bruce Stillman

Chairpersons

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Mr. George F. Ohrstrom
Dr. and Mrs. James M. Stone

Members

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Mr. and Mrs. Raymond Wong

On October 17–18, Bruce Stillman hosted CSHL's annual President's Council Fall Meeting. This year's meeting focused on plants, biofuels, and the future of energy.

(*Left to right*) President Bruce Stillman with President's Council Co-Chair John Friedman and John Hofmeister, former President of Shell Oil Company, at the 2008 President's Council Fall Meeting.

CSHL plant scientist Rob Martienssen (*left*) with President's Council member Ira Hazan.

Cold Spring Harbor Laboratory Association (CSHLA)

We are grateful to members of the Cold Spring Harbor Laboratory Association who came together often in 2008, cultivating new friends and raising unrestricted funds in support of CSHL scientists. Despite the difficult economy, members raised over $1.1 million for the annual fund.

Here, we mention a few highlights from a busy year: "The Lab Goes Latin" event last April was a lively change from our traditional spring jazz benefit. We applaud Lisa and Tim Broadbent and Kate and John Friedman for the tremendous energy and creativity that they put into this successful event. In early June, lots of new friends hosted dinner parties for visiting and CSHL scientists after the annual Symposium's Dorcas Cummings lecture about skin stem cells, delivered by Elaine Fuchs, Ph.D., of The Rockefeller University. A rainy Friday evening in September was no deterrent to the record number of our major donors who enjoyed a lovely reception hosted by CSHL trustee Stephen Lessing and his wife Sandra in their beautiful home.

The year 2008 closed with the completion of Pien Bosch's role as President of the Association Board. We could not be more grateful for her exceptionally dedicated service to CSHL. Pien's devotion to CSHL's far-reaching goal to improve human health resulted in a welcome influx of friends and supporters who share her sense of privilege in having a world-renowned institution in "our backyard."

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We are lucky to have high-level support from two advisory bodies: the Education Committee and the Corporate Advisory Board (CAB). The Education Committee formulates policy and assists with strategic planning, while the CAB provides liaison to the Long Island business community. The CAB also conducts an annual golf tournament and the annual fund campaign. These are the DNALC's major sources of unrestricted funding. In 2008, more than $275,000 was raised through these events.

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Eric Krasnoff (*left*), Chairman and CEO of Pall Corporation, with friends at the 15th annual DNALC Golf Tournament

CSHL President Bruce Stillman and Christine McInerney of the Jerome L. Greene Foundation with a student at the newly established *Harlem DNA Lab*

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The third annual Double Helix Medals Dinner was held on November 6, 2008 at the Mandarin Oriental in Manhattan. Pictured are 2008 Double Helix Medals recipients Jim Watson (*left*) and J. Craig Venter (*center*) with Deborah Norville and CSHL President Bruce Stillman.

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(*Left to right*) J. Craig Venter, Bill Harman, and CSHL Honorary Trustee David Koch at the Double Helix Medals Dinner

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2008 Double Helix Medals recipient Sherry Lansing with CSHL President Bruce Stillman (*left*) and Herb Siegel

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On November 1, 2008, a dedication of the Laurie J. Landeau Multimedia Studio was held at the Dolan DNA Learning Center. Jim Watson (*left*) is pictured with CSHL Trustee Laurie Landeau, DNA Learning Center Executive Director Dave Micklos, and CSHL President Bruce Stillman.

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(*Left to right*) Tom Calabrese, Judy and Pat Woods, and Trudy Calabrese at The Lab Goes Latin fundraiser

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(*Left to right*) John Habich Solomon, CSHL Trustee Andrew Solomon, and CSHL Chairman Emeritus Jim Watson at the Double Helix Medals Dinner

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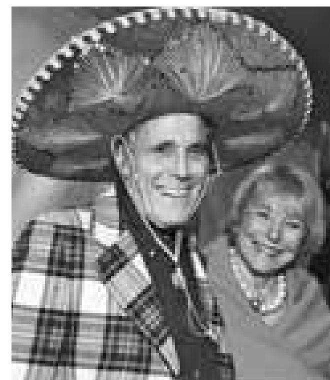
CSHL Trustee Don Axinn with his wife Joan at The Lab Goes Latin fundraiser

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CSHL Scientist Mikala Egeblad (*center*) accepts a check from Long Island 2-Day Walk to Fight Breast Cancer.

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