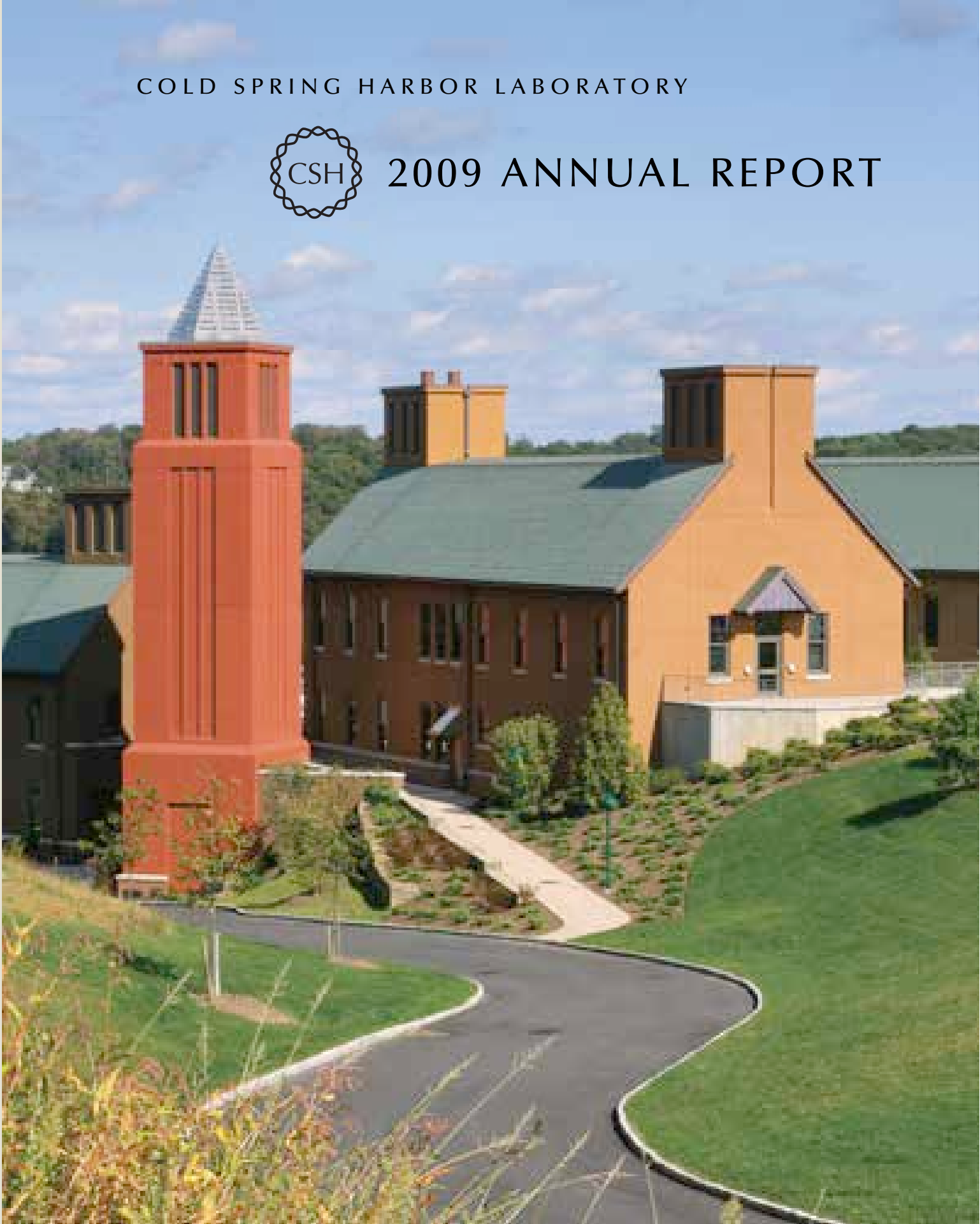
COLD SPRING HARBOR LABORATORY

2009 ANNUAL REPORT



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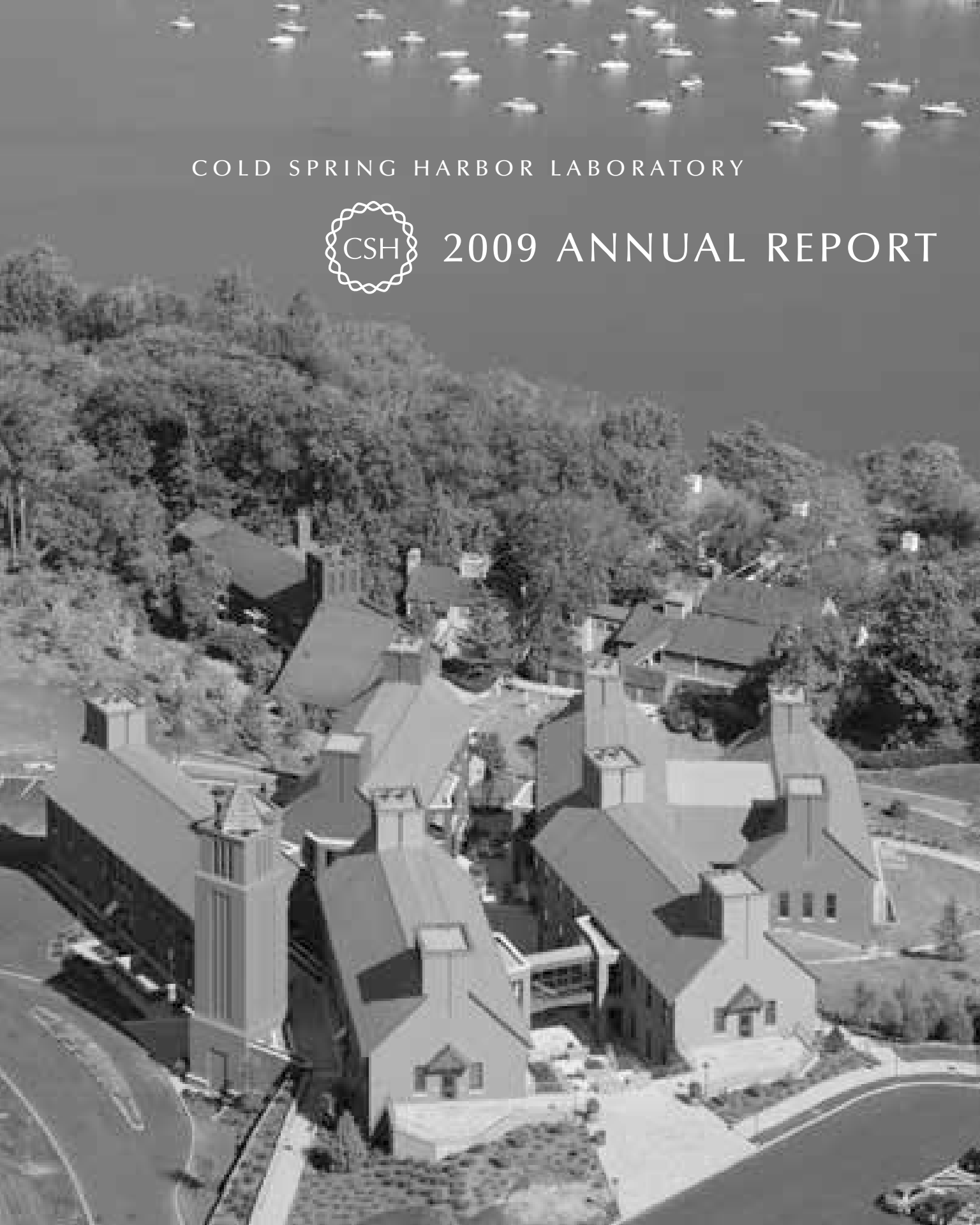

ANNUAL REPORT 2009

Cold Spring Harbor Laboratory
One Bungtown Road
Cold Spring Harbor, New York 11724

www.cshl.edu

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Front cover: North view of the six new Hillside Research Laboratories. Photo by Jeff Goldberg/Esto.

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*Deceased Oct. 2009
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*Deceased Oct. 2009
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Donald Everett Axinn (1929–2009)

Donald Everett Axinn was one of the quiet Long Islanders who had a major impact on the well-being of the communities and institutions that make up much of what Long Island is about. Cold Spring Harbor Laboratory was fortunate to have him as a dear friend and benefactor and suffered a great loss when he lost his battle with cancer at the age of 80. From the day he was introduced to the Laboratory via our President's Council and as an early indirect investor in some of our spin-off companies, Don displayed a keen interest in our science and its relationship to people's lives. He also fostered the mingling of literature and science via his passion for writing.

Founder and chairman of the Donald E. Axinn Companies, an investment and real estate development firm, he served on the CSHL Board of Trustees since 2006. Don was active in the building, finance, and nominating committees, where he shared his finely tuned business and real estate development skills and experiences to benefit CSHL. Don was also generous in sharing his friends with CSHL, and along with his wife Joan, he enriched our connections to many new supporters. Most importantly, he helped our chief facilities officer Art Brings with the completion of the new Hillside Laboratories, one of the largest projects ever completed on our main campus. Don appreciated the integration of artistic and functional elements of design that are clearly visible in the six buildings that collectively formed the heart of the project. Hard-hat on, he patrolled the construction site reviewing plans, questioning craftsmen, negotiating with vendors. He took it personally, and through his involvement became ever more integrated into the CSHL family. We were honored by the pride that he took in the successes of this institution, which he publicly proclaimed to all who would listen.

Don's pride in the institution and these new laboratories was such that he very generously financially supported The Donald Everett Axinn Laboratory, one of the six research buildings that constitute the visually elegant complex. Adjacent to the Axinn Laboratory is the Laurie and Leo Guthart Discovery Tower, honoring Don's long-time friend, business associate and fellow Laboratory Trustee Leo Guthart. Don and Leo together supported a major cancer research effort in Greg Hannon's laboratory.

It was particularly pleasing to see Don and Joan, joined by family and friends on June 12, 2009, for the Hillside Laboratories dedication and that he was able to celebrate this monumental achievement with all of us. We captured his zest for adventure in the sky in a beautiful portrait of Don and his 1944 Boeing N253 Navy Stearman painted by local artist David Peikon. A passionate airman and strong supporter of the Long Island Cradle of Aviation Museum, Don helped to envision the eight exhibit galleries housed in the Donald Everett Axinn Air and Space Museum Hall.

Born of Russian émigré parents, Don graduated from Middlebury College where he later received an honorary Doctor of Letters, one of five such degrees that honored his major accomplishments. He maintained a strong association with Middlebury, as a writer in residence and supporter of the college's Donald E. Axinn '51 Center for Literary and Cultural Studies at the Starr Library.

Don served on Long Island's Hofstra University Board of Trustees from 1970 to 1979, including terms as Secretary and Vice Chair. He did an amazing thing in 1971–1972 when he assumed, on a pro-bono basis, the Associate Deanship of the Hofstra College of Liberal Arts and Sciences. He rejoined the Board at Hofstra as an active trustee in 1984, serving until 1990 when he became a Trustee Emeritus. In 1988, the Hofstra University Library was named the Joan and Donald E. Axinn Library in recognition of his many accomplishments and his service as a valued advisor to the University.

Don was known as the quintessential 20th-century renaissance man, pursuing three distinct careers. In addition to his accomplishments as a successful real estate developer, he was an accomplished writer and made a lifelong commitment to public service via philanthropy.

Altogether, Don wrote eight volumes of poetry and three novels. One of them caused Don to become a film producer when his novel *Spin* was made into a movie by director James Redford, winning a Crystal Heart Award at the Heartland Film Festival. Don was a Tennessee Williams Fellow at the Bread Loaf Writers' Conference, and we were proud to host poetry readings by Don as part of the annual CSHL Cultural Series. He generously donated the proceeds of his latest novel *Allan, Burning* to CSHL. In reviewing this latest novel, Nelson DeMille wrote, "Donald Axinn brings his renowned poetry to this beautifully written story of a man in crisis... . [T]his is a novel that will make you think and compel us all into re-examining our ordinary lives."

In addition to supporting research at CSHL, Don and Joan devoted themselves to environmental and humanitarian causes. Don was one of the founders of the Interfaith Nutrition Network, which provides shelter and kitchens for the homeless on Long Island. He also served as a director and/or trustee for numerous public organizations, among them The Nature Conservancy, Poets & Writers, The Academy of American Poets, and Friends for Long Island's Heritage.

Don Axinn continues to fly high above us.

Bruce Stillman

A poem that Don wrote for us hangs in the Axinn Laboratory building.

THE COLORS OF INFINITY

beyond cold and comprehension
past stashed universes
where god contemplates
the shapes of cosmic islands where the
dead have escaped
old bones and cacophony

where I almost remember
the tingle of the first kisses
my children's uncorrupted faces
slipstreams and winging
into the white mystery of clouds
the musty smells of august's forest floors
smoke and wildflowers

there in that wondrous place
where night and day
are always the same
where the end or beginning
never matter
there I would bathe in the strong
rich colors of infinity

John Cleary
(1933–2009)

John Cleary's death from a heart attack while on a skiing holiday in Colorado was a grievous blow not only to his family but also to Cold Spring Harbor Laboratory and to his many close friends on Long Island. His calming, intelligent personality made us increasingly dependent on his skills as a consummate lawyer and counsel. He helped to steer me in the new world of biotechnology and later into the world of high-powered publishing and entertainment. The coming of the 50th anniversary of the double helix had created a potential need for a well-crafted TV series and attendant book that would let the general public appreciate the ever-increasing relevance of DNA to our daily lives.

John understood all too well the importance of genetics in the diagnosis and treatment of human disease. Too soon after the birth of his and Rita's youngest son, Jimmy, weakness in his leg muscles had led to the diagnosis of Duchesne muscular dystrophy (DMD), an affliction due to mutational changes in the gene coding for vital muscle protein dystrophin. After arrival of recombinant DNA technologies made possible the cloning of disease genes, John became hopeful that some clever gene therapy technique would cure his son. Unfortunately then and even today, the problem of how to deliver good dystrophin genes to diseased muscle cells remains beyond human capability.

Temporarily I held out hope to John and Rita that Jimmy's disease might be curable if one could find drugs that would greatly increase the synthesis in muscles of eutrophin, a relative of dystrophin just discovered in Oxford by Kay Davies. With monies provided by the farsighted head of the French Muscular Dystrophy Society, Long Island's new biotech start-up Oncogene Sciences (now OSI Pharmaceuticals) screened its own proprietary chemical libraries for compounds that would turn on eutrophin synthesis. Unfortunately, no such molecules were found.

John became the first investor for Mirus Bio, of Madison, Wisconsin, founded on the principle that nonviral (i.e., plasmid-DNA-based) methods offer distinct advantages over viral methods of gene transfer. Following this path, Mirus has pioneered the development of a wide range of nonviral delivery technologies using proteins, polymers, and lipids in conjunction with novel chemistries that provide unique nucleic acid delivery capabilities. John served on its board until its growing success led to its acquisition by Roche Pharmaceuticals in 2008.

Increasingly aware of recombinant DNA's unique potential for producing much needed new drugs, John became committed to the nurturing of a cluster of biotech companies in the center of Long Island. Without John's leadership, the Broadhollow Bioscience Park at SUNY Farmingdale would not exist. He was personally involved in securing local and statewide support from the New York State Legislature, the State University of New York, and key state employees. Most appropri-

ately as the first chairman of its board, he worked to bring Cold Spring Harbor Laboratory and SUNY Farmingdale together to encourage biotech start-ups.

John's formal involvement with Cold Spring Harbor Laboratory began as a Director of the CSHL Association in 1988, serving as its President from 1994 to 1997 and at the same time also serving on the CSHL Board of Trustees. From 2001 to his untimely death on February 1, 2009, he was appointed an Honorary Trustee, regularly attending its meetings and remaining a sage source of common sense. While on the board, he served on the Executive Committee, the Building Committee, the Development Committee, and the Community Relations Committee. Always aware of the Lab's need for new monies to expand into new frontiers, John with Rita made significant gifts to the Double Helix Medals dinner, the Women's Partnership lunch, the Mary Lindsay Child Care Center, and the Watson School of Biological Sciences through establishing the John P. Cleary Visiting Lectureship. Their gift to the Campaign for the Hillside Laboratories let us recently dedicate the John P. and Rita M. Cleary Lounge in the Les and Jean Quick Laboratory for Cancer Research.

After graduating from Yale University in 1954 with a B.A. in Economics, he earned his law degree from New York University Law School in 1961. John was a founding partner of Farrell, Fritz, Caemmerer, Cleary, Barnosky and Armentano in Uniondale, where he is remembered as a man of his word with ethics beyond reproach. A trustee of Molloy College from 1979 to 1997, he served as its Chairman from 1985 to 1998. His long service to Molloy was recognized in 1998 when the college awarded him an honorary LLD.

Through his passing, the Cold Spring Harbor Laboratory lost a passionate voice for biomedical research. We will very much miss his reassuring presence.

James D. Watson

PRESIDENT'S REPORT

As the year covered by this report came to a close, we reached the end of the new century's opening decade. In the brief interval of 10 years, the American economy soared, crashed, and then soared and crashed again. And yet here at Cold Spring Harbor Laboratory, we remained on a remarkably even keel, blazing a trail forward on multiple fronts. One of the virtues of scientific research is that it does not come to an abrupt halt in hard times. Without a doubt, we are able to do more when all of the economic arrows are pointing upward. But it is a measure of the strength of our institutions that progress in science does not correlate, at least over the short-run, with the condition of the economy.

The challenges posed by the current recession have been by no means trivial. We have made a concerted effort to husband our resources and reduce costs while keeping at full strength the superb faculty and staff that has put us at the leading edge of discovery. Our base of private donors has risen to the occasion, as has the federal government, which wisely set aside additional funds for scientific research as part of a broad economic recovery and revitalization package. Our scientists performed admirably in the ensuring competition for one-time special funding.

The Laboratory has not simply weathered the storm—it has flourished. Our campus has not contracted along with the economy during the last year but rather has grown with our burgeoning research capabilities. The magnificently designed Hillside Laboratory buildings opened in June and provided our faculty with ~40% more space in which to perform their work. We will be adding faculty as funding permits, thereby enhancing our already world-class programs in cancer, neuroscience, and plant genetics research, as well as building a new center of expertise in the field of quantitative biology.

All of this is possible because of groundwork we laid in the first years of the decade. Extensive planning, coupled with both public and private generosity, enabled a plan made in boom times to come to fruition during a trough. Although I am fond of pointing out that science moves inexorably forward, it is important to qualify that science of the caliber performed at Cold Spring Harbor Laboratory is possible only because of careful forethought, periodic assessment of trends in research, and philanthropy that drives innovation.

As I survey the road ahead, I would be remiss if I did not offer some cautionary words. Although academic science has flourished during the past decade, as powerful new technologies have enabled us to reap the fruits of the many genome sequences that have been determined, the private sector has lagged behind. Ever since the technology bubble broke at the beginning of the decade, funds for value-added science have all but disappeared, and in the last few years, venture capital investment in the biomedical sciences has dropped precipitously. By value-added science, I mean the kind of research and development activity that adds commercial value to discoveries made by scientists engaged in basic research. Value-added science was once the purview of early-stage biotechnology companies. In the current environment, however, such companies are not being formed. Compounding the problem, pharmaceutical investment in research has also dropped, owing to the recessionary economy and a reduction in corporate income as major pharmaceuticals have gone off patent.

During the past decade, scientists at Cold Spring Harbor Laboratory have developed a rich collection of technologies for discovery and validation of new therapeutic targets, particularly for cancer. This followed from a deliberate expansion of our research into areas that are closer to the clinic, yet still strongly rooted in basic discovery. In the coming years, we shall increasingly be contributing to knowledge that is of great potential value to projects of national and global import, particularly in health-related sciences. But there are other areas in which biology can make a major impact.

This past year, the National Academy of Sciences Board on Life Sciences, of which I am a member, issued a report from a committee cochaired by Thomas Connelly of DuPont Company and Phillip A. Sharp, CSHL alumnus, MIT professor, and Nobel laureate. The report identified four grand challenges to which 21st-century biology might contribute in major ways: cultivation of food plants that will sustain

the growing global population, development of methods with which to sustain ecosystem function and biodiversity, creation of sustainable and economical alternatives to fossil fuels, and continued progress toward medicines that are increasingly coupled to individual experience and genetics.*

If what the National Academy committee calls the "new biology"—collaborative, multi- and cross-disciplinary science—is to rise to these challenges, I believe we who perform basic science and guide it at the institutional level will have to address one internal challenge and advocate for action on a related, but external, front.

The problem we must address within basic science concerns our relationships, individually and collectively, with private industry. The ability of academia and industry to collaborate has become clouded by controversy arising from recent disclosures of unreported financial arrangements made between private-sector companies, notably large pharmaceutical firms, and a small number of scientists and physicians. What can only be regarded as the undeclared greed of a tiny minority of academic scientists now imperils the health of the research establishment as a whole. Following the uncovering of a few cases in which clinicians received large sums of money from industry, sums deliberately hidden from their academic institutions, and later, from a Congressional investigation of certain medical schools, the entire academic community was tarred with the same brush. Some members of Congress have now caused the National Institutes of Health (NIH) to introduce regulatory reforms that are adding more detailed layers of reporting of conflict of interest. Remarkably, those that violated the previous and reasonable institutional policies have, as far as I know, retained their academic positions. Clearly if the few clinicians who blatantly violated their intuitional policies had been appropriately disciplined, we might have avoided the now more burdensome regulations and scrutiny that have been imposed. It is now the situation that if an academic scientist has a relationship with a company, motives are immediately suspect.

The solution is obvious. Full disclosure must be made by research scientists (and immediate family members) of all external financial arrangements directly pertinent to their work, including ownership of company shares, as well as fees from speaking engagements, board service, and the like. This was the situation in all leading research centers before the recent abuses came to light. Now, reports must be made to the federal government, with clarifying follow-up questions that seem to me to be feeding a bureaucracy and not achieving any real purpose. For example, in advising the National Cancer Institute, I have found the level of disclosure and documentation of personal information required before every meeting to be excessive. I know of senior scientists who have been counseled not to join such advisory committees because of the potential criminal liability that is coupled with the requirements for accuracy of such detailed disclosures. The process has gone too far, as such measures now stand as a disincentive for well-meaning people to provide advice to the scientific community. The mere fact of disclosure—publication over the Internet and in publications—will serve to curtail abuses. Beyond the humiliation that will be visited upon any faculty transgressor, I believe properly documented abuses should be punished by dismissal or by preventing transgressors from applying for public research funds.

At the same time, it is vital that regulators not choke off the vitality of research activity. Overreaction would almost certainly have a harmful impact upon discovery and innovation nationwide. Indeed, there is nothing wrong if basic scientists who make significant discoveries to which commercial value is subsequently added, in the process of translation or commercialization, are able to benefit from their contributions. Appropriately, as is now the case, most of the financial benefits that may derive from commercial research in academia do not go to individual scientists but to the institutions in which they work, so that it can be plowed back into more research. The public interest will in fact be damaged if scientists and institutions are discouraged from thinking about practical applications of their own research.

Those who make discoveries in basic science ought not be prevented or scared away from entering into partnerships with industry. Rather, they should be encouraged to do so, particularly if we are to accomplish the grand challenges that the National Academies report has identified for biology. Collaboration between academia and industry is also needed if we are to fulfill the promise of basic research under way

**A New Biology for the 21st Century: Ensuring the United States Leads the Coming Biology Revolution*. National Research Council, The National Academies, Washington, D.C.

today at Cold Spring Harbor Laboratory: cataloging genes and tracing pathways involved in major mental illnesses, as prelude to the development of diagnostics and next-generation treatments; acquiring a much deeper understanding of metastasis in cancer and the means of predicting and circumventing resistance to chemotherapy as a means to identify new therapeutic strategies; laying the groundwork for the therapeutic use of gene-regulating RNA interference; and applying results from experimentation in plant development to processes such as increasing food production and generating biofuels.

We must also advocate for a solution on a second and related front, equally important to ensure the vitality of basic science and its translation to value-added research. Since the steep stock market decline of 2002–2003, private financing for new science-based companies has virtually dried up. Venture capital is not being risked as much as it was in the past, and the market for initial public offerings for companies that do get started has essentially ground to a halt. Such an economic climate has greatly reduced a major source of funding that supported value-added science. One mechanism to overcome the steep decline in small-company formation is for industry and academic interactions to be made more seamless than they are now. It is important that publication of basic research results upon which applied science is necessarily based not be delayed by discussions about intellectual property even before such property has been developed. I suggest that improved interactions between academia and industry may be the only way to benefit from the sizable public funding of research in the United States. Industry must find new ways to develop research interactions with academia that are more open and collaborative, and academic institutions must limit their appetite for financial benefit, a concern that often drives them to extended negotiations about intellectual property and financial reward.

If we are to spur translation of basic biological discoveries, we need to identify better models for academic–industry collaboration. Such measures could include venture capital investment in research within academic institutions so that venture capital does not have to bear the substantial costs associated with establishing a completely independent entity until it is obvious that the research warrants it. Perhaps a review of policies concerning the Small Business Innovation Research (SBIR) grants provided by NIH at the request of Congress will provide funds for new modes of collaboration. These might enable venture capital and government to better collaborate in seeding early-stage value-added research within academia before companies exist.

Alternatively, large existing corporations and academia should discover new ways of interacting. Cold Spring Harbor Laboratory now has an interesting partnership between our plant geneticists and the Pioneer Hi-Bred division of the DuPont Company. This entirely above-board relationship represents the best of two worlds: our basic research and Pioneer's product development engine. Laboratory scientists collaborate with company scientists on research of mutual interest. Our scientists benefit from access to resources that only could be provided within a large corporation, such as the rapid mapping of gene mutations in plants, and company scientists become involved in exciting early-stage research that normally would not be possible if they were left to work on their own within a corporate development environment. Early access to new discoveries should enable Pioneer to capitalize on those ideas, ensuring that there is a path to commercialization for our basic research. We both benefit, as will society, if, for instance, new methods of boosting crop yields are the result. Such arrangements should not be frowned upon as benefiting industry with taxpayer funds, because the United States economy depends on the success of such advancements. Otherwise, we will find that published research from United States–based academic institutions will be commercialized in other countries.

To sum up, scientists must fully and publicly disclose all arrangements from which they stand to benefit, transgressors should lose their jobs or their ability to seek taxpayer-supported research funds, scientists should be able to profit from their inventions, and academia and industry must be encouraged to invent new ways to interact that will ultimately serve the public good.

It would be a supreme irony if Americans regulated themselves out of a world-leadership position in the sciences. Responsibly managed, basic science can and should provide fuel for entrepreneurialism in America, a signature attribute of the nation that links scientific discovery with public benefit.

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

Research

Good scientists know how to judge the relative contributions of their colleagues, peers, and institutions, mostly via a subjective assessment of research. Indeed, it is part of the process of recruiting new faculty; early-career scientists are judged on their potential for an outstanding research career, and Cold Spring Harbor Laboratory (CSHL) is known as a place in which such young people excel. There are, however, some objective criteria, such as the number of times research papers are cited by colleagues, indicating on average a relatively high impact.

This year, Thompson Reuters, a science publisher well known for its Essential Science Indicators, again placed CSHL atop a list of 20 "heavy hitters" in molecular biology and genetics selected from a database comprising more than 42,000 research institutions worldwide. This particular measure of impact, covering the last 10 years, was based on the number of times, on average, papers written by a given institution's faculty were cited by their peers. Other institutions in the top 20 were Massachusetts Institute of Technology, Salk Institute for Biological Studies, Memorial Sloan-Kettering Cancer Center, The Rockefeller University, and Harvard University. Other rating organizations also place CSHL at the very top of research institutions worldwide, and CSHL has consistently been placed as number one in these ratings for the past three decades. These ratings are not the only measure of research impact, of course, and we do not use such information when assessing the progress and promotion of our individual scientists. But in general, the rating does reflect the view I have long held of our institution, based on intimate first-hand knowledge. An exciting research agenda is part of what makes CSHL a great place to work. In 2009, our scientists were as productive as ever, a fact reflected in the highlights of some of the research that appear below.

CSHL ranked number one for worldwide impact in molecular biology and genetics

Mouse Models of Leukemia That Predict Human Response to Chemotherapy

This past year, Scott Lowe and colleagues developed new mouse models for human acute myeloid leukemia (AML), a devastating cancer of white blood cells. Most patients with AML receive intense chemotherapy followed by additional chemotherapy cycles or bone marrow transplantation; only a quarter of patients are cured and most die within a few months. The range in treatment response is due to AML's genetic heterogeneity, meaning that the 100 or so mutations associated with this form of cancer occur in different combinations in each patient and influence therapeutic outcomes in different ways. Scott's group identified the most commonly occurring mutations in a sample of 111 children with AML and then engineered these mutations into mice, which soon developed leukemia. Of the two most common mutations they observed, one, in an oncogene called *AML1/ETO*, previously had been associated with a favorable therapeutic outcome in people; the other, in an oncogene called *MLL,* was associated with an adverse outcome. To design an animal model that predicts these outcomes, the team introduced each mutation individually into stem and progenitor cells along with another oncogene, called Nras, which also appears frequently in human AML and is commonly found in concert with *AML1/ETO* and *MLL* oncogenes. These altered stem cells were transplanted into mice pretreated with radiation to destroy existing bone marrow cells. The altered stem cells then took over the "host" bone marrow and promoted the development of leukemia, which, within weeks, showed the same genetic and pathological features as human AML. Just as in humans, leukemias in mice that received the *AML1/ETO* oncogene were also sensitive to chemotherapy and soon regressed, whereas *MLL*-triggered

S. Lowe

leukemias remained resistant and eventually killed their hosts. These findings suggest that such models can predict how human cancers will respond to therapy and help to identify genes promoting resistance or sensitivity to any cancer drug. The mouse models also serve as an effective test system for new drugs and treatment strategies. Indeed, CSHL Fellow Chris Vakoc, in collaboration with Scott's laboratory, is now searching for new therapeutic targets for the *MLL* form of leukemia.

A Protein That Blocks Progression of Malignant p53-deficient Tumors

More than half of all human cancers have mutations that disable a protein called p53. The product of a master tumor-suppressor gene by the same name, p53 is central in several cancer-fighting operations within cells. When cells lose p53, tumors grow aggressively and often cannot be treated. But this past year Alea Mills and colleagues demonstrated that there is a chink in the armor of p53-deficient tumors—a protein called TAp63, the product of a gene called *p63*, which Alea discovered as a postdoctoral student a decade ago. The *p63* gene is usually intact and not mutated in most cancers. Mills and her team succeeded in shutting off growth in tumors in which p53 is missing by turning up the production of TAp63 proteins; it completely blocked tumor initiation by inducing senescence, a state of growth arrest in which tumor cells are still metabolically alive but fail to divide. More importantly, turning up the levels of TAp63 in p53-deficient cells blocked the progression of established tumors in mice. As Alea suggests, this means that we now have a model of how to attack refractory human cancers that have damaged p53.

A. Mills

A Drug Candidate for Treating Spinal Muscular Atrophy

Spinal muscular atrophy, or SMA, is a devastating illness, and although quite rare, it is nevertheless the leading genetic cause of death in infants. It is caused by mutations in a gene called Survival of Motor Neuron 1 (*SMN1*), which cause levels of SMN protein in the motor nerve cells of the spinal cord to diminish. These nerve cells, or neurons, are the cells that control muscle activity, and without the protein, they degenerate. Infants born with *SNM1* mutations progressively lose the ability to move, swallow, and breathe. There are no approved therapies for SMA. This year, we were excited to learn that Adrian Krainer's lab, in collaboration with colleagues at Paratek Pharmaceuticals and Rosalind Franklin University of Medicine and Science, has identified a tetracycline-like compound that stimulates SMN production by altering RNA splicing. Called PTK-SMA1, it is the only small molecule known to specifically alter RNA splicing by directly and solely targeting the splicing reaction. The team confirmed that the effect of PTK-SMA1 on RNA splicing and exon inclusion ultimately results in increased levels of full-length and functional SMN protein. The compound boosted protein levels in cells isolated from SMA patients and cultured in lab dishes. The team also proved its ability to work in vivo by injecting it into mice carrying a human *SMN2* gene. The mice showed a more than fivefold increase in human SMN protein levels within a week of treatment. Adrian and colleagues will next tackle the questions of how PTK-SMA1 redirects RNA splicing and will seek a way of getting it across the blood-brain barrier and into affected neurons in the spinal cord. His laboratory is also working on other therapeutic strategies for SMA that look very promising

A. Krainer

Small RNAs That Protect the Germline

CSHL is at the forefront of research on small RNAs. Two fascinating studies published by our faculty this past year reveal the vital role played by these tiny bits of nucleic acid in the defense of the germline, in very different species. Rob Martienssen led a team that looked at sperm cells in plant pollen grains. In this setting, sperm are cocooned within larger "companion" cells, called pollen vegetative cells. It has long been known that the companion cells provide sperm with energy and help move them to their targets during fertilization. Rob's team determined that they also provide sperm with instructions that protect their DNA from damage and thus help pass on a stable genome

R. Martienssen

to the next generation. The instructions offered to sperm specifically come in the form of small RNA molecules that companion cells pass on to sperm. These small RNAs can inactivate, or "silence," specific DNA sequences. In this way, they help set up gene expression patterns in sperm, providing the next generation with instructions that specify which regions of the genome should be turned on and which should be switched off and protect the sperm from expressing genes that might be detrimental when the pollen fertilizes cells for the next generation.

A separate study by Greg Hannon and colleagues examined how the germline in fruit flies is protected from genetic parasites called transposons. These bits of DNA sequence have infiltrated host genomes over the eons and can cause damage by copying and inserting themselves in random fashion across genomes, disrupting genes and regulatory sequences. To protect themselves from transposons, animal germline cells have developed a molecular immune system, operated by an army of small RNA molecules called Piwi-interacting RNAs (piRNAs) and a set of proteins belonging to the Piwi family. Greg's team discovered that in the ovaries of fruit flies, nongermline, or "somatic cells," that surround germline cells have also developed an antitransposon defense system. Over the years, fruit fly researchers have uncovered genomic mutations that lead to sterility and abnormal development. Because these defects could have been caused by unchecked transposon activity, mutant flies are a good experimental resource to uncover exactly how piRNA pathways work and how they might get disrupted. Hannon's team analyzed eight such mutants, showing how the genes disrupted in each mutant impact the piRNA pathway and how it alters the type and number of piRNAs that cells are able to generate. These studies help us understand the broad picture of how the piRNA pathway has been genetically stitched together to perform its vital role in protecting the germline and genetic information that will be passed from parents to the next generation.

G. Hannon

Mobile Small RNAs That Set Up Leaf Patterning in Plants

Anyone who has taken the time to carefully inspect a plant leaf knows that the top and bottom surfaces are not quite the same. In fact, this difference is the product of a developmental program that establishes an asymmetry crucial for the leaf's function: It ensures that the leaf develops a flattened blade optimized for energy production by photosynthesis, with a top surface specialized for light harvesting and a bottom surface containing tiny pores that serve as locales for gas exchange. Plant scientists have known that the top/bottom axis is established by a signal derived from the meristem, the stem cell-rich growing tip of the plant from which all new leaves arise. Other signals that traffic between the upper and lower sides of the leaf are thought to stably maintain this polar axis. In 2009, Marja Timmermans and her team were the first group to uncover the identity of one such positional signal—a family of mobile small RNAs generated on the upper surface of young leaves but which traffic to form a concentration gradient across each leaf. This graded distribution pattern of small RNA molecules creates discrete regions of gene activity so that cells in each half of a leaf develop a distinct "top" or "bottom" identity. Besides providing a remarkable example of a morphogen-like small RNA signal, Marja and her team have also shown that the location of the various biochemical ingredients required for small RNA activity can impact pattern formation. Together, their discoveries explain how mobile small RNAs can generate leaf patterns during development.

M. Timmermans

Identification of a Protein That Enhances Long-term Memory by Controlling Rest Periods

Students everywhere—those who study, at any rate—know from experience that studying improves memory, but only under certain conditions. Facts are preserved longer in memory if a student spaces out learning sessions between rest intervals. This past year, Yi Zhong and his team discovered how


Y. Zhong

this so-called "spacing effect" is controlled in the brain at the level of individual molecules. Yi has long been interested in genes that when mutated trigger learning and memory disorders such as Noonan's syndrome, a rare genetically inherited disease. More than half of Noonan's patients have mutations in a gene called *PTP11*, which encodes the SHP-2 phosphatase protein. In contrast to many disease-related mutations that shut off protein production or impair protein activity, these *PTP11* mutations do the opposite—they boost the activity levels of SHP-2 phosphatase. To understand how this change impedes long-term memory, Zhong's team engineered these mutations into a gene in fruit flies called *corkscrew* that is the functional equivalent of *PTP11* in humans. The team found that normally, as each learning period ends, SHP-2 phosphatase activity inside stimulated neurons triggers a wave of biochemical signals, which have to peak and decay before the next learning session can begin. They discovered that the repeated formation and decay of the biochemical signal during each rest interval induces long-term memory. In normal flies, these signal waves took 15 minutes to peak and decay. In the mutants that had excess protein activity, however, the signaling wave took 40 minutes to decay. This research shows it is crucial that the period of rest should last as long as it takes for a signal wave to form and reset. Yi's team succeeded in reversing memory deficits in mutant flies, by reducing the activity of mutated SHP-2 phosphatase to normal levels with drugs or simply altering training regimens to include 40-minute rest intervals instead of the normal 15 minutes. These results suggest a potential means with which to address memory impairments in an illness such as Noonan's syndrome.

Structure of the NMDA Subunit Reveals Target for Drugs Against Neurological Diseases

Hiro Furukawa and colleagues obtained crystal structures this year for one of several subunits of the NMDA (*N*-methyl-D-aspartate) receptor. This receptor type is one of a family that mediates excitatory transmission in nerve cells in the brain. One theory of causation in Alzheimer's, Parkinson's, and multiple sclerosis posits that excessive amounts of the excitatory neurotransmitter glutamate can cause an overstimulation of glutamate receptors, including the NMDA receptor. Such excitotoxicity, the theory holds, can cause nerve cell death and subsequent neurological dysfunction. The search is well under way for molecules that can shut down the NMDA receptor. To do so in a highly specific manner—one that would potentially carry lower risk of unwanted side effects—we need a precise map of the receptor and its active sites at the level of individual atoms. Hiro's team focused on a portion of the extracellular domain of the receptor, a subunit called NR2B. It includes a domain of particular interest called the ATD (the amino terminal domain), whose structural distinctiveness makes it a potentially attractive target for future drugs. Hence, the importance of the team's achievement: A crystal structure revealed by the powerful light source at nearby Brookhaven National Laboratory that shows the ATD to have a clamshell-like appearance that proves to be important for its function. Work can now proceed on rational design of a drug that can precisely bind the ATD within what Hiro and colleagues call its "clamshell cleft," based on the crystal structure they have obtained.


H. Furukawa

Roles of a Key Protein, Associated with Mental Retardation, on Both Sides of the Synapse

This past year, Linda Van Aelst and colleagues demonstrated the mechanism by which a signaling protein found throughout the brain controls the maturation and strength of excitatory synapses, the tiny gaps across which the majority of neurons communicate. The discovery is important, in part, because deficits of the signaling protein in question, called oligophrenin-1 (OPHN1), have previously

L. Van Aelst

been associated with X-linked mental retardation. Indeed, problems at the synapse—in their formation and in the mechanisms through which the strength, or plasticity, of their connections are regulated—are thought to contribute to numerous mental and neurological disorders. Linda points out that at least 280 genes have already been implicated in metal retardation. But what we have not done, to date, is connect the genetic abnormalities to biological processes that establish and modify the function of neuronal circuits. Previously, Linda had shown that dendritic spines—knoblike structures that protrude from a neuron's branch-like dendrites and receive signals across synapses from the axons of other neurons—are short and misshapen when expression of the *OPHN1* gene is acutely reduced. Her team's new experiments showed that the OPHN protein is not essential for the formation of dendritic spines, but it is needed for the proper maintenance of their structure. Importantly, in this maintenance function, the OPHN1 protein was found to have a key role both in the maturation of excitatory synapses and in their plasticity, or ability to vary in strength. In related experiments, focusing on the presynaptic side of the gap between nerve cells, they found that OPHN1 also helps neurons to transmit messages, by controlling the recycling of synaptic vesicles in presynaptic terminals. This suggests that symptoms of X-linked mental retardation could stem not only from having both immature and deformed dendritic spines, but also from inefficient neuronal vesicle retrieval and recycling.

A Reference Genome for Maize

In late 2009, a 4-year, multi-institutional effort co-led by three CSHL scientists culminated in publication of a landmark series of papers in the journal *Science* revealing in unprecedented detail the DNA sequence of the maize plant. Maize, or corn, as it is commonly called in North America, is one of the world's most important plants and the most valuable agricultural crop grown in the United States, representing $47 billion in annual value. The sequence spans 2.3 billion DNA base-pairs and contains ~32,500 genes, or about one-third more than the human genome. This version of the maize genome—taken from a variant called B73—is regarded by the scientific and agricultural communities as a reference version. Doreen Ware, one of the CSHL coprincipal investigators, contributed important annotation and evolutionary analysis. In a parallel effort, Doreen's CSHL team also helped generate a draft haplotype map of maize, in collaboration with the USDA. As in humans, the maize HapMap gauges genomic diversity by comparing distinct individuals—in this case, 27 maize lines—with the reference version. Dick McCombie and Rob Martienssen were the other CSHL coprincipal investigators on this important project. Having the sequence and a HapMap of this critical plant will enable scientists to find genes associated with quantitative traits—genes that affect traits of importance to agriculture, everything from the size of the seeds to when the plant flowers to whether it can tolerate drought or dampness. This may help plant scientists develop maize varieties that will thrive as the planet warms in the period ahead.

D. Ware

R. McCombie

Likely Origin of Facial Cancer Decimating the Tasmanian Devil Population

An international team led by Greg Hannon and his former student, Elizabeth Murchison, of CSHL and the Australian National University, succeeded in identifying the likely point of origin for the deadly facial tumors decimating Australia's Tasmanian devil population: Schwann cells, cells of the nervous system which form a tissue type that cushions and protects nerve fibers. The discovery stems from the team's effort to carry out a genetic analysis of tumor cells in devil tumor facial disease. DFTD is a unique type of cancer transmitted from animal to animal via biting or other physical contact. Tumors in the canine-sized devils are mostly found on the face and mouth, but they often spread to internal organs. With no diagnostic tests, treatments, or vaccines currently available, the aggressive disease could wipe out the Tasmanian devil species, which is found only on that island-state of Australia, in 25 to 35 years. The largest surviving marsupial carnivores, the devils have become a cause célèbre for conservationists worldwide. Greg and his team determined the identity of the originating cell by using advanced sequencing technology to uncover the tumors' transcriptome—the complete set of genes that are turned on in tumor cells. Comparing this readout to that from other tissues, they found that the tumors' genetic signature best matched that of Schwann cells. Armed with the tumors' genetic profile, researchers now can start hunting for genes and pathways involved in tumor formation. A catalog of devil genes compiled by the Hannon–Murchison team should be useful in designing vaccines and other therapeutic strategies.

Cold Spring Harbor Laboratory Board of Trustees

The Board of Trustees elected three new members this year: Michael R. Botchan, Ph.D., Goldman Professor and Chair of the Department of Molecular and Cell Biology, University of California, Berkeley, and a former faculty member at CSHL; Thomas Quick, President of First Palm Beach Properties, Inc., who begins a second period as Trustee; and Samuel L. Stanley, Jr., M.D., the fifth president of Stony Brook University.

In addition, the Board named Nancy Marks as an Honorary Trustee. Nancy served on the board as a Trustee from 2004 to 2009 and participated in the Development Committee (2004–2006), the Capital Campaign Committee (2006–2008), and the Building Committee (2000–2009).

Congratulations to CSHL Scientific Trustee Charles L. Sawyers, M.D., chair of the Human Oncology and Pathogenesis Program at Memorial Sloan-Kettering Cancer Center, who in September received the 2009 Lasker-DeBakey Clinical Medical Research Award for groundbreaking work on the treatment of chronic myeloid leukemia.

CSHL mourned the passing of Honorary Trustee John P. Cleary, Honorary Trustee, early in the year. John and his wife Rita made significant philanthropic contributions toward the research and education mission of the Laboratory, recently supporting the expansion of CSHL's research infrastructure and the creation of the Broad Hollow Bioscience Park to promote biotechnology research on Long Island.

The CSHL community also grieved for Trustee Donald Everett Axinn who died in November 2009. The Donald Everett Axinn Laboratory opened this year as part of the largest construction project ever undertaken by CSHL. The successful completion of this building project was in large part due to Don's support and guidance.

I thank the CSHL Board of Trustees for its leadership and generosity in the successful completion of the Capital Campaign, which began in 2005 with a goal to raise $200 million to realize a 40% expansion of the Laboratory's research capacity. With significant contributions from our Trustees, we surpassed the original goal and raised $340 million over the period of the Capital Campaign. In June 2009, we celebrated the dedication of the six new buildings that make up the new Hillside Laboratories.

The Cold Spring Harbor Laboratory Association, under the leadership of president Tim Broadbent, had a successful year, raising $4.8 million of critical unrestricted funds to support early-career scientists at CSHL. Events that contributed to this achievement included the Double Helix Medals

Dinner, which alone raised more than $3 million; the President's Council, which raised over $375,000; and the Women's Partnership for Science luncheon, which raised close to $50,000. The balance was contributed by CSHL Association members.

On behalf of CSHL, our Board of Trustees, and our Development Department, I thank all those who helped us achieve our goals. Private philanthropy is the engine of innovative research, and your contributions are pushing the boundaries of science forward. Please refer to the back of this Annual Report for a complete list of our generous supporters.

Research and Education Management

Our research and education management teams performed exceedingly well in the face of the challenges that the world financial crisis presented. CSHL's investigators and administrators worked closely to effectively manage existing programs under conditions where we had to cut our budget mid year. In an unprecedented team effort, CSHL secured more than $22 million in federal stimulus grants issued under the American Recovery and Reinvestment Act (ARRA). These 2-year funds will support research in cancer, neuroscience, epigenetics, and plant biology, as well as research training and laboratory enhancements.

In applying for research grants, applicants were encouraged to develop innovative and bold ideas in relatively short grant proposals. CSHL scientists had a 30% success rate in securing ARRA grants, much higher than the national average. I suspect that this is because much of our innovative science is supported by philanthropy or by internal endowment funds, and our scientists are used to proposing bold ideas. If such proposals were submitted in normal individual research grant proposals (the so-called RO1 mechanism), such ideas would invariably be shot down and not funded. Perhaps this is a lesson of how the National Institutes of Health (NIH) should consider funding some science in the future.

True to CSHL's legacy as a breeding ground for the latest technologies and approaches to solving biological questions, CSHL secured special 5-year grants for "transformative" research projects. Our researchers Josh Dubnau, Ph.D., and Partha Mitra, Ph.D., received these grants for neuroscience projects that the NIH deemed "exceptionally innovative, high-risk, original, and/or unconventional… [with] the potential to create new or challenge existing scientific paradigms."

We were also encouraged by a pledge of continued support to stem cell research from New York Governor David A. Paterson, who visited CSHL in October with State Health Commissioner Richard F. Daines, M.D., in order to be briefed on stem cell research projects by CSHL investigators Drs. Linda Van Aelst, Grigori Enikolopov, Marja Timmermans, and CSHL Clinical Fellow Dr. Johannes Zuber.

The CSHL Scientific Advisory Council (SAC) met for the first time this year in March, lending third-party scientific expertise to help CSHL continue to maintain its global leadership position in

Govenor Patterson visits with stem cell researchers

Scientific Advisory Committee members and CSHL leadership. (*Left to right*) David Spector, Tony Pawson, Cornelia Bargmann, David Botstein, Carol Greider, Markus Meister, Joanne Chory, Bruce Stillman, Max Wicha, Frederick Alt, Leonid Kruglyak, and Sydney Gary

research and education. To provide the SAC with requisite background, the CSHL leadership devoted a number of sessions to a broad overview of the research, environment, and philosophy of CSHL. Several other sessions were devoted to discussions of specific questions and topics that CSHL senior administration had provided in advance of the meeting. In addition, SAC members met with both postdoctoral fellows and graduate students and held a session open to all CSHL faculty. The meeting ended with the SAC members providing helpful recommendations and feedback on specific issues related to research at CSHL. I thank the SAC Chair Fred Alt and all the members of the committee who provided very valuable advice.

CSHL Education Programs

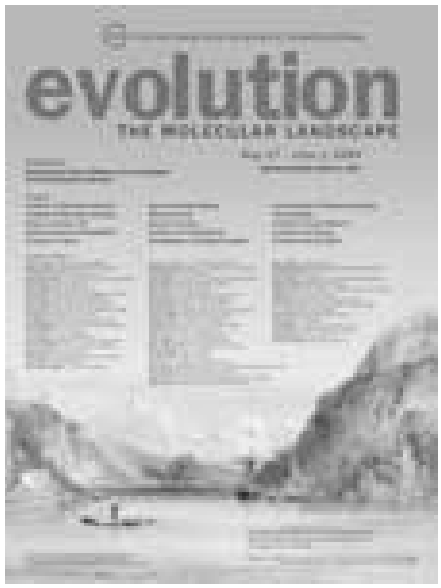
CSHL's Watson School of Biological Sciences (WSBS) marked its first decade on April 26, graduating five new Ph.D.s: Allison Blum, Daniel Chitwood, Shu-Ling Chiu, Keisha John, and Jeremy Wilusz. SarahJane Locke received the Master of Science degree. This year brings the total number of WSBS graduates thriving in the outside world to 35. I am particularly pleased that even in its short history, six graduates of the school already have faculty positions at major universities or research institutes, supporting our thesis that the path to scientific independence need not be long.

WSBS honorary degree recipient Winship Herr, Ph.D., commemorates the 10-year anniversary of the establishment of the Watson School

The 2009 Commencement Convocation ceremony also conferred honorary degrees upon individuals who have made remarkable contributions to CSHL's wide-ranging and innovative education programs, including the Dolan DNA Learning Center, the Undergraduate Research Program, the CSHL Press and our advanced courses, and the Watson School of Biological Sciences: David Micklos, Alfred Goldberg, Jeffrey Miller, and Winship Herr, each of whom has made remarkable contributions to educational programs at CSHL.

CSHL's advanced scientific course offerings expanded into new facilities that were supported by the Howard Hughes Medical Institute. This new laboratory enables our advanced courses to expand teaching on brain anatomy and neural networks, cognition and behavior.

The 74th CSHL Symposium, "Evolution: The Molecular Landscape," celebrated Charles Darwin's 200th birthday and the 150th anniversary of his revolutionary work, *On the Origin of Species*. Approximately 400 scientists gathered at this 6-day-long conference to discuss a wide range of evolution-



74th CSHL Symposium

Suzhou Dushu Lake Conference Center auditorium

A courtyard within the Dushu Lake campus

themed topics, ranging from the origins of life on a molecular scale to the emergence of species both simple and complex over the last three billion years.

The reputation of CSHL's Meetings and Courses Program continues to grow, as evidenced not only by attendance, which reached a record of 6500 this year, but also by external, independent ratings. The September 2009 edition of the magazine *Genome Technology* ranked CSHL's "Biology of Genomes" meeting as "the most recommended" among general genomics meetings. Another CSHL meeting called "Genome Informatics" was the "most recommended" in the Bioinformatics/ Information Technology category.

Cold Spring Harbor Laboratory Conferences Asia convened its first meeting in Suzhou, China in November. This invitation-only Banbury-style meeting was held in temporary facilities while our purpose-built conference center was being completed. The meeting focused on transgenic crops and served as a prelude to the opening of a complete program of large-scale meetings on a wide range of topics in the biological sciences in 2010. The $70-million 600,000-square-foot conference center can accommodate up to 500 participants.

The Dolan DNA Learning continues to blaze new trails in web-based educational experiences. This year, DNALC's BioMedia Group launched "Genes to Cognition Online" (www.g2conline.org), which is distinguished by both its content and its presentation on the web. The site uses a unique approach to depict the complex and interlocking relationships between different aspects of brain anatomy and function. Just as the brain itself is composed of interconnected networks of cells, the site graphically represents information about these components as members of a vast network, whose nodes are interconnected.

DNALC three-dimensional brain iPhone app

The BioMedia Group also produced an exciting *iPhone* application that can be download for quick and easy access to a three-dimensional model of the brain and its functions. Rapidly, this application became one of the top educational tools downloaded to *iPhones.*

The CSHL Press published *Cold Spring Harbor Perspectives in Biology*, a new online publication spanning the complete spectrum of the molecular life sciences. Each issue includes reviews covering a wide variety of topics in molecular, cell, and developmental biology, genetics, neuroscience, immunology, cancer biology, and molecular pathology. Contributions are written by leading researchers in each field and commissioned by a board of eminent academic editors.

Awards and Honors

Many of CSHL's younger researchers received prestigious awards this year, recognizing their early-career accomplishments. Adam Kepecs was made a Klingenstein Fellow in Neurosciences and was also named an Alfred P. Sloan Research Fellow. Adam's laboratory is combining its behavioral expertise with molecular and optical techniques to monitor and manipulate genetically identified circuit elements in behaving mice. Bo Li received a Dana Foundation Award to investigate how hyperactive brain synapses may be key to understanding depression and formulating a treatment.

A. Kepecs

B. Li

Z. Lippman

P. Osten

Zach Lippman won a Human Frontier Science Program Career Development Award to continue his work in understanding the molecular dynamics that underlie altered developmental fates of certain plant meristems. Pavel Osten received the McKnight Technological Innovations in Neuroscience Award for his use of novel imaging technology to map changes in neural circuits in mice that carry genetic mutations linked to autism and schizophrenia. Grisha Enikopolov was the recipient of the Ellison Foundation Senior Scholar Award.

Lin He, a former CSHL postdoctoral fellow was named a MacArthur Fellow by the John D. and Catherine T. MacArthur Foundation. She was honored for advancing our understanding of the role of microRNAs in the development of cancer and laying the groundwork for future cancer treatments.

Former CSHL Fellow and Faculty member Carol Greider, along with her colleagues Elizabeth Blackburn and Jack Szostak, won the 2009 Nobel Prize for Physiology or Medicine for discovering how chromosomes are protected during cell division by telomeres and the enzyme telomerase. During her fellowship from 1988 to 1990, Dr. Greider identified the RNA component of the enzyme telomerase that adds DNA to the ends of chromosomes. She continued her research career as a member of the CSHL faculty from 1990 to 1997 and is currently Daniel Nathans Professor and Director of Molecular Biology and Genetics at the Institute for Basic Biomedical Sciences at Johns Hopkins School of Medicine.

C. Greider, winner of the 2009 Nobel Prize for Physiology or Medicine

Development

For the eighth consecutive year, CSHL earned a four-star rating for sound financial practices from the philanthropic evaluator Charity Navigator. Only 1% of the approximately 5000 nonprofit organizations analyzed by Charity Navigator have achieved this milestone.

Building Projects

Congratulations to the entire team of CSHL Facilities Department staff and the many Long Island craftsmen and women who were part of the Hillside Laboratories complex project. The construction of the largest building project in CSHL history was completed this year—on schedule and within

CSHL received Charity Navigator's highest rank for the eighth consecutive year

budget. Commissioning of the Hillside Laboratory buildings was accomplished, with the new Simons Center for Quantitative Biology and the relocated operations of the CSHL Cancer Center occupying finished space. The Hillside Laboratories complex also contains an additional animal facility that was brought on line in 2009. The CSHL Information Technology Department and an updated and expanded datacenter were relocated to the Hillside complex. This is the new home of the High Performance Computing Center (HPCC)—CSHL's very own supercomputer.

For 120 years, CSHL has been a proud steward of the Long Island shoreline and local ecosystem. The design and construction of the Hillside Laboratories demonstrates our continued commitment to the environment. The new facilities were designed to be 30% more efficient than standards set by the American Society of Heating, Refrigeration, and Air-Conditioning Engineers (ASHRAE). With help from the Long Island Power Authority (LIPA), CSHL successfully reduced the environmental footprint of the new facilities and reduced energy costs associated with facility operations. The total cost of electrical energy efficient measures incorporated into the project was $1,057,022, which was offset by a $224,940 Commercial Construction rebate from LIPA.

The Hillside Laboratory complex also breaks ground architecturally. The project won the Platinum Award from the American Council of Engineering Companies of New York for Excellence in Engineering for outstanding design. The awards ceremonies were held in March 2010 at the Waldorf Astoria Hotel in New York City.

Hillside Laboratories

Campus view from Cold Spring Harbor Village

In addition to the new construction on the upper campus, we completed scheduled improvements to the ca. 1927 Delbruck Laboratory building's historic teaching lab space. The project, which was made possible by funds from the Howard Hughes Medical Institute, included reconstruction of the top floor and roof of the building and the renovation and expansion of an existing conference room.

Across the harbor at the Banbury Conference Center in Lloyd Harbor, we also completed the interior renovations to the ca. 1937 Robertson House, which provides lodging for visiting scientists who attend Banbury meetings and participate in CSHL's advanced courses on the latest scientific technologies and techniques. Installation of modern HVAC, electrical, and data systems now make the manor house comfortable for guests throughout the entire year.

The reconstruction and addition to the Carnegie Building, which is home to the CSHL Library and Archives and The Genentech Center for the History of Molecular Biology, was largely complete by the end of the year. We look forward to the official reopening of the building in the spring of 2010.

At the Genome Center in nearby Woodbury we constructed a new, state-of-the-art greenhouse to allow for an expansion of our plant biology program that is being led by a new faculty member, Zachary Lippman, Ph.D., who studies varieties of tomato plants to understand the mechanisms that control flower, fruit, and seed production.

To increase operational efficiency across the expanding Laboratory, we are leasing a facility in Syosset that allows us to centralize receiving, storage, and fulfillment operations. This facility also provides needed office and administrative space.

Special Events

- **Gavin Borden Visiting Fellows.** The 15th Annual Gavin Borden Visiting Fellow Lecture, in memory of the publisher of *Molecular Biology of the Cell*, was held on March 24. The lecture was presented by Ralph J. Greenspan, Senior Fellow in Experimental Neurobiology, Lewis B. and Dorothy Cullman Senior Fellow, The Neurosciences Institute, San Diego, California.
- **Symposium.** During the 74th Symposium, "Evolution: The Molecular Landscape," the traditional Dorcas Cummings Memorial Lecture for scientists and guests from the community was delivered by Kevin Padian, Professor of Evolutionary Biology and Paleontology, University of California, Berkeley. The title of the lecture was "Darwin, Dover, and Intelligent Design."
- **Women's Partnership for Science.** On June 14 at Peacock Point, at the Lattingtown home of Mr. and Mrs. Daniel P. Davison, nearly 140 women lunched and learned about the link between viruses and cancer, specifically human papillomavirus (HPV), a prime cause of cervical cancer. The speakers included the Dean of the Watson School of Biological Sciences and Howard Hughes

Greenhouse at the Woodbury Genome Center

Medical Institute Investigator, Leemor Joshua-Tor, Ph.D., and Felicia Callan, M.D., obstetrician/gynecologist at the Mount Sinai School of Medicine/North Shore Medical Group. Money raised at this event supports women who pursue careers in biomedical research at CSHL.

L. Joshua-Tor

- **Hillside Laboratories Opening.** On June 12, CSHL dedicated the Hillside Laboratories, with remarks from Chairman of the CSHL Board of Trustees Eduardo Mestre; Chancellor Emeritus Jim Watson, Ph.D.; Bill Grover, FAIA, founding partner of Centerbrook Architects and Planners; and myself. The keynote address, "Thoughts on the Future of Biological Sciences," was delivered by Philip A. Sharp, Ph.D., Nobel laureate, University Professor, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology.
- **The President's Council.** CSHL Board Chairman Eduardo Mestre and his wife Dr. Gillian Shepherd hosted an April 16 reception to announce the theme of the members-only 2009 President's Council program: "Personal Genomes." Special guests at this Manhattan event were Linda Avey and Anne Wojcicki, co-founders of the genome sequencing company 23andMe. The annual fall President's Council retreat was held on October 16-17 and featured Peter Neufeld, co-founder and -director of The Innocence Project. Other speakers that weekend included: David Botstein, a geneticist and CSHL Scientific Trustee; Esther Dyson, whose own genome was among the first sequenced in the Personal Genome Project; Elaine Mardis, Co-Director of The Genome Center, Washington University School of Medicine; Dr. Philip Marshal of WebMD Health Services; and CSHL Assistant Professor Gurinder "Mickey" Atwal.
- **The Double Helix Medals Dinner.** The 4th Double Helix Medals Dinner was held at the Mandarin Oriental Hotel in Manhattan on November 10. Medals for Scientific Research were presented to Herbert W. Boyer, Ph.D. and Stanley N. Cohen, M.D., who co-discovered recombinant DNA. Life-long philanthropist and advocate for research Kathryn W. Davis, Ph.D., was honored for Humanitarianism. In recognition for his unprecedented support of biomedical research, Maurice "Hank" Greenberg was presented with the medal for Corporate Philanthropy. Violin virtuoso Joshua Bell performed with accompaniment by pianist Frederic Chiu. The event was cochaired by Mr. and Mrs. Eli Broad, Mr. and Mrs. Christopher Davis, Ms. Florence A. Davis, Mr. and Mrs. Edward E. Matthews, and Dr. Richard H. Scheller.

Double Helix Medal

President's Council members Thomas Lehrman, Kristina Perkin Davison, Judy Carmany, and George Carmany (*front row, left to right*) discuss personal genomes

Double Helix Medals Dinner at the Mandarin Hotel, Manhattan

- **The Lorraine Grace Lectureship on Societal Issues of Biomedical Research.** On November 29, science journalist Nicholas Wade, Ph.D., presented the first annual lecture, introducing his newly published book, *The Faith Instinct—How Religion Evolved and Why It Endures*.

CSHL Public Lectures

March 23—William C. Mobley, M.D., Ph.D.: *The Future of Down Syndrome: Improving Memory and Cognition*, sponsored by the National Down Syndrome Society and the Down Syndrome Connection of Long Island.

April 27—Josh Dubnau, Ph.D., CSHL Assistant Professor: *Memories of a Fly: What a Fly's Brain Can Teach Us About Our Own.*

May 26—Darwin Commemorative Lecture: Sean Carroll, Ph.D., Professor of Molecular Biology, Genetics and Medical Genetics, University of Wisconsin, Madison: *Remarkable Creatures: Epic Adventures in the Search for the Origin of Species.*

September 17—Rob Martienssen, Ph.D., CSHL Professor: *Designing Bioenergy Crops: Developmental Problems and Genetic Solutions.*

October 13—Scott Lowe, Ph.D., CSHL Professor and HHMI Investigator: *Cancer Research at Cold Spring Harbor Laboratory: Our Latest Successes and the Road Ahead.*

October 22—Senthil Muthuswamy, Ph.D., CSHL Assistant Professor: *Breast Cancer Research in 3-D: A New Way of Looking at How Tumors Develop.*

Sean Carroll, author of *Remarkable Creatures,* presented the Darwin Commemorative Lecture at CSHL

CSHL Public Concerts

April 17—Irina Muresanu and Dana Ciocarlie, violinist and pianist

May 1—Michelle Cann, pianist

May 15—Frederic Chiu, pianist

September 4—Margarita Shevchencko, pianist

September 11—Steven Beck, pianist

September 25—Ken Noda and Tamara Mumford, pianist and mezzo soprano

October 2—Inbal Segev, Dmitri Berlinsky, and Elena Baksht, cellist, violinist, and pianist

October 9—Daria Rabotkina, pianist

Laboratory Employees

New Staff

S. Shea

This year, we welcomed two new Assistant Professors: Stephen Shea and Mikala Egeblad. Stephen Shea received his Ph.D. from University of Chicago in 2004 and completed his postdoctoral fellowship at Duke University. Stephen studies the neural circuitry of social communication and decisions. His laboratory's efforts are directed at answering a number of related questions such as: What are the neural mechanisms for interpreting social information and selecting appropriate behaviors? How do we discriminate and remember familiar individuals? How do emotion and social context shape our attention and memories? And how are social cues from our various senses integrated to shape behavioral decisions? Stephen directly addresses these questions using natural social communication behavior in mice.

M. Egeblad

Mikala Egeblad joins us from University of California, San Francisco, where she was an Assistant Researcher. Mikala received her Ph.D. from the University of Copenhagen and the Danish Cancer Society in 2000. Her work addresses the challenge of separating functions and behaviors of the different stromal components of the tumor. In her lab, she uses mouse models of breast cancer and powerful real-time imaging of cells in tumors in live mice. This enables her to follow the behaviors of and the interactions between cancer and stromal cells in tumors during progression or treatment.

Promotions

A. Krasnitz

Congratulations to Marja Timmermans, Ph.D., who was promoted this year to full professor, and to Alexander Krasnitz, Ph.D., who was promoted to assistant professor.

Departures

In 2009, several faculty members left CSHL for appointments in other parts of the country. Jacek Skowronski is now a professor at Case Western Reserve University, School of Medicine. William Tansey is a professor at Vanderbilt University. Tim Tully is Chief Science Officer at Dart Neuroscience.

Community Outreach

In addition to the Cultural Series of concerts and lectures for the public that we organize each year in cooperation with local organizations, CSHL continues to be an active participant in the community here on Long Island. We are also becoming an increasingly visible part of science-related action in New York City.

2009–2020 Partners for the Future with CSHL faculty

Last year, our campus welcomed 30 community-based groups as part of a program that is staffed by a very energetic team of CSHL students and postdocs. Thanks to them, attendance in our regularly scheduled Saturday morning walking tours for the public had a waiting list! The Dolan DNA Learning Center's hands-on Saturday programs and summer camp sessions continue to sell out.

Seven Long Island high school seniors recommended by their school's science chairs were selected for this year's Partners for the Future program at CSHL. These promising young people worked hand-in-hand throughout the school year on real experiments with some of CSHL's top scientists: Drs. Tony Zador, Josh Huang, Michael Zhang, Alea Mills, Doreen Ware, and David Jackson. In close collaboration with the Cold Spring Harbor School District, CSHL participated in this community's first American Cancer Society "Relay for Life" event, with remarks from CSHL Director of Research David Spector and Dr. Michael Wigler. CSHL students, postdocs, and researchers volunteered at several science fairs judging the projects of middle and high school students across Long Island. CSHL students and postdocs also hosted local elementary school students, teachers, and parents for a field trip.

Spearheaded by Harlem DNA Lab instructor Ileana Rios, CSHL participated in the second annual World Science Festival (WSF) in Manhattan, serving as a laboratory workshop for high school kids participating in the festival's "Pioneers in Science" Program. Highlights of the event included a demonstration of banana DNA extraction at a street fair in Washington Square that drew a total crowd of 100,000 New Yorkers! Jim Watson was also involved in the WSF this year, as part of the gala and as a central figure in a performance by artist Anna Deavere Smith in "Watching Watson and Wilson," a one-woman show composed of vignettes that portray Jim and E.O. Wilson.

CSHL employees volunteer at local science fairs

Community Support

Each year, our employees demonstrate their commitment to the local community in so many ways. We held three blood drives on campus this year in February, August, and October. This effort set a record, collecting 20% more blood in 2009 than in 2008.

We continue to participate in the Secure the Call Foundation's effort to convert used cell phones to 911 emergency-use phones for those in

DNALC instructor Ileana Rios at the World Science Festival Street Fair in Manhattan

need. CSHL volunteers prepared and served dinner at the Ronald McDonald house to 40 families of seriously ill children. We also collected 300 pounds of food in support of the Long Island Cares Harry Chapin Food Bank.

Looking Forward

The financial and economic setbacks in 2008–2009 will most likely cause a major change in the long-term prospects for both philanthropic and federal support of science. We can be secure that our science continues to be world leading and hence will attract support, but increasingly in tight times, we must be aware that both members of Congress and taxpayers are increasingly looking at the outcomes of basic research. The economic impact of research is obvious, but changing how we interact with industry is going to be necessary if we are to achieve these goals. More fundamentally, we must increase the applied value of our research internally. Finding a mechanism of funding to do this will create a major challenge in the future.

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

CHIEF OPERATING OFFICER'S REPORT

W. Dillaway Ayres Jr.

In a recent *New York Times* op-ed column entitled "The Big Zero," Nobel Prize–winning economist Paul Krugman presented his assessment of the performance of the U.S. economy during the first decade of the new millennium. Lamenting the fact that during the 10 years, there was zero growth in stock and home values after inflation, and observing that the dot-com bubble was quickly followed by the housing bubble, he dryly concluded that this was "the decade in which we achieved nothing and learned nothing."

Happily, the same cannot be said for Cold Spring Harbor Laboratory. In the last 10 years, we have built the Freeman Building, the Marks Laboratory, and the Hillside Laboratory complex as well as acquired and renovated the Woodbury Genome Center and the Syosset Center, thereby adding 170,000 square feet of research space and 40,000 square feet of support space. Major renovations and additions to many facilities have taken place including the Dolan DNA Learning Center, James Laboratory, Harris Research Support Facility, Robertson House, and Carnegie Library. An elite and highly successful graduate school with a student body of 50 Ph.D. students was founded and built. The Meetings & Courses and Dolan DNA Learning Center educational programs have developed overseas operations. Our faculty has expanded in number from 35 to 50 while the overall employee population has grown from 700 to more than 1000. At the same time, our operating income and total assets have doubled to $129 million and $650 million, respectively. In addition to a steady annual fund-raising program, two major campaigns were initiated and completed: the $30 million Watson School endowment campaign in 2000 and the $200 million campaign to support the Hillside Laboratories in 2008. As testament to the fact that the growth in both program and plant did not come at the expense of academic excellence and fiscal prudence, the Laboratory has been rated the number-one research institution in the country for productivity over the decade and its credit rating has been upgraded to a very solid AA– status.

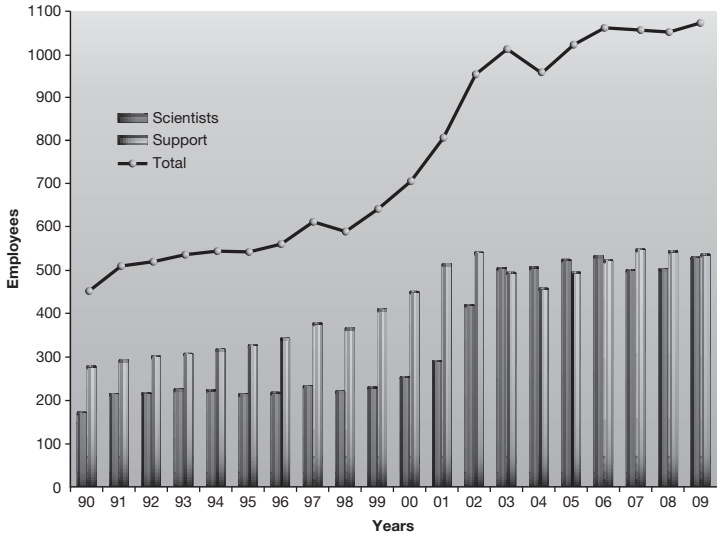
All of this is not to suggest that the recent financial crisis and recession have not inflicted institutional pain. As was reported last year, although our endowment fund outperformed that of the average university, it nevertheless declined 24% during the 2008 calendar year. The year 2009 began just as poorly with the S&P 500 Index down 6% in the first quarter. Fortunately, the market began to recover in March and along with it, the value of the Laboratory endowment. The portfolio returned 20% for the calendar year—a relatively strong performance given that 30% of the fund was conservatively allocated to fixed-income investments. Total market value at year end increased to approximately $250 million, although still well short of its high-water mark. To position the portfolio to achieve solid growth while reducing volatility, the Investment Committee of the Board of Trustees has reduced exposure to "long only" equity managers while increasing the allocation to alternative and hedged managers who are less closely correlated to market indexes.

For the fiscal year ended 12/31/09, the Laboratory operating budget reached approximately $129 million—a 4% increase over the prior year. Due to the practice of calculating annual endowment spending as a percentage of a moving average of market value over the previous 12 calendar quarters, we knew that the steep decline in the fund value in 2008 would put substantial pressure on our 2009 operating budget. In fact, the endowment spending draw in 2009 was nearly $3 million less than in 2008. Consequently, we fell somewhat short of our goal of achieving cash-neutral operating results after netting depreciation expense against expenditures on capital improvements.

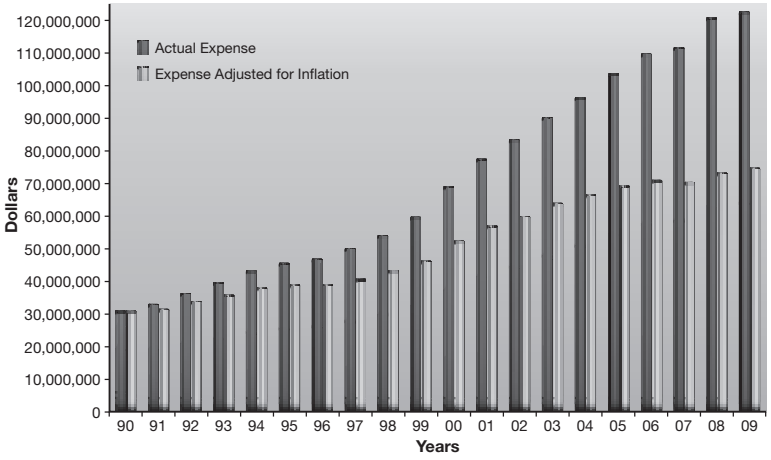
Looking forward to 2010, there is reason for both optimism and caution. The Laboratory's investigators have been extremely successful in securing new grants that became available as part of the American Recovery and Reinvestment Act—driving a substantial increase in projected program

Staff

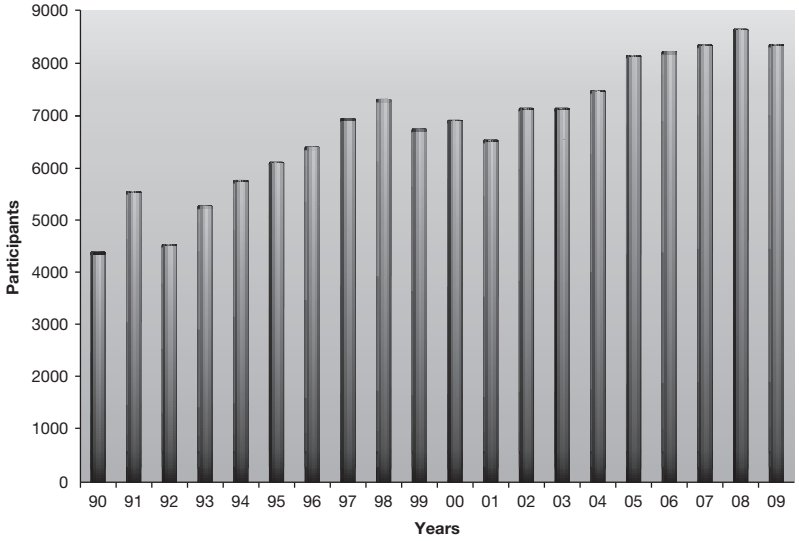
(Consists of full-time and part-time technical support, core services, publications, meetings, library, public affairs, buildings and grounds, administrative, personnel, Banbury Center, and DNA Learning Center)



Operating Expense



Meetings and Courses Participants



revenue. However, many of these are temporary "stimulus" awards, are mostly limited to 2 years in duration, and the revenue will need to be replaced in 2012. Expenses continue to be diligently managed and we are encouraged by an opportunity to significantly reduce energy costs in the coming years. Finally, we continue to get strong support from our private donor base despite the trying economic times. This is critical because the future of the institution is dependent on rebuilding and growing our endowment funds.

We are profoundly grateful to our loyal supporters and to our talented and dedicated staff.

Dill Ayres
Chief Operating Officer

Long-term Service

(*Back row*) Drew Mendelsohn, Danny Miller, Jennifer Troge, Dill Ayres, Jan Eisenman, James Watson, Bruce Stillman, Dessie Carter, Spencer Teplin, David Spector, Yuri Lazebnik, Jim Hope; (*middle row*) Idee Mallardi, Carmelita Bautista, Maureen Berejka, Alison Mc Dermott, Mary Ellen Goldstein; (*front row*) Jim Bense, Sharon Bense, Carol DuPree, Susan De Angelo, Janet Argentine, Judy Cuddihy, Jamie Bonilla, Leigh Johnson, Diane Esposito, Rob Gensel, John Meyer

The following employees celebrated milestone anniversaries in 2009:

35 Years	Lane Smith
30 Years	Maureen Berejka, Judy Cuddihy, Jim Hope, John Meyer, Bruce Stillman
25 Years	Carmelita Bautista, Dessie Carter, Rob Gensel, Mary Ellen Goldstein, Danny Miller, Steven Tang
20 Years	Jim Bense, Sharon Bense, Charlene De Poto, Jan Eisenman, Leigh Johnson, Rob Martienssen, Jackie Matura, Alison Mc Dermott, Spencer Teplin
15 Years	Leslie Allen, Janet Argentine, Jaime Bonilla, Susan De Angelo, Carol DuPree, Diane Esposito, Yuri Lazebnik, Philip Lembo, Rob Lucito, Idee Mallardi, Drew Mendelsohn, Sharon Story, Jennifer Troge, Tracy Woolfson

RESEARCH

See previous page for photos of the following scientific staff:

Row 1: G. Atwal (Atwal Lab); E. Vernersson Lindahl (Mills Lab); H. Oviedo (Zador Lab); A. Campbell (Turner Lab)

Row 2: C. Ruse (Pappin Lab); D. Albeanu, A. Dhawale (Albeanu Lab); Y.-T. Yang (Van Aelst Lab); T. Caulfield (McCombie Lab)

Row 3: Z. Lippman, S. Park (Lippman Lab); L. Joshua-Tor, R. Prakash (Joshua-Tor Lab); W. Li, J. Dubnau (Dubnau Lab); K. Revill (Powers Lab)

Row 4: J. Taranda, P. Osten (Osten Lab); N. Cutter (Lucito Lab); A. Krainer, M.A. Jensen (Krainer Lab); R. Burgess (Hannon Lab)

Row 5: E. Karakas, N. Simorowski, H. Furukawa (Furukawa Lab); A.M. Naguib, C. Pratt, L. Trotman (Trotman Lab); L. Lintault, L. Dow (Lowe Lab); A. Goldschmidt (Jackson Lab)

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.

Thomas Gingeras and colleagues study where and how functional information is stored in genomes. These efforts 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 ENCODE (ENCyclopedia of DNA Elements) and modENCODE (model genome ENCODE) projects of the National Institutes of Health. His research has revealed that almost the entire lengths of genomes in organisms ranging from bacteria to humans, contrary to expectations, can be transcribed into RNA and that most RNA products are not destined to be translated into proteins. Rather, noncoding RNAs (ncRNAs) are proving to be involved in a variety of other important biological functions, only a small fraction of which are known. Some ncRNAs have been shown to be critical components in the pre- and posttranscriptional and translational processes. Others serve as scaffolds upon which large protein complexes are assembled. This year in a collaboration with Greg Hannon, Gingeras and colleagues discovered a new posttranscriptional processing pathway culminating in the generation of capped short RNAs, which provide a record of transcribed regions and some spliced RNA sequences.

Gregory Hannon is a pioneer in the study of RNA interference (RNAi) in mammalian systems. In RNAi, double-stranded RNA molecules induce gene silencing. In addition to elucidating key elements of the RNAi machinery, Hannon and colleagues have led the way in using RNAi to study cancer biology and genetics, generating a library of short hairpin RNAs that researchers at CSHL and elsewhere apply broadly in gene-silencing studies. This year, the laboratory reported results in a number of concurrent projects. They announced their discovery in fruit flies of an antitransposon defense system in somatic cells that is entirely distinct from that previously seen in germline cells. The system, they found, guards against a particular class of transposon called *gypsy* elements. In a collaboration with Thomas Gingeras, Hannon discovered a new posttranscriptional processing pathway leading to the formation of a new class of small RNAs, called non-PSARs, which, clipped from mature protein-coding RNAs, can regulate gene expression. The lab introduced a method of harnessing the logic of Sudoku math puzzles to vastly enhance genome-sequencing capability; it is being used to analyze the genomes of large populations to identify individuals who carry mutations that cause genetic diseases. The lab also had a critical role in the work of an international team which discovered that Schwann cells are the probable cellular origin of the deadly transmissible facial tumors decimating Australia's Tasmanian devil population.

The laboratory of Leemor Joshua-Tor focuses on protein complexes involved in nucleic acid regulatory processes. They use X-ray crystallography to obtain three-dimensional structures of individual proteins and atomic-level views of their interactions with other molecules. Joshua-Tor is well known for her work on the helicase enzyme, which acts to unwind DNA strands during the DNA self-replication process, and for revealing structures that help explain the gene-silencing mechanisms of RNA interference. This year, Joshua-Tor and colleagues helped to explain the mechanism by which heterochromatin is assembled. Heterochromatin is a highly compressed bundling of chromosomal material such that genes in these areas are rendered inaccessible and therefore "silent." Joshua-Tor's team demonstrated that heterochromatin assembly depends on the strength with which a protein called Chp1 binds to a specific target site located on a histone protein attached to the double helix.

Adrian Krainer's lab studies mechanisms of RNA splicing, 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 neuromuscular disease that is the leading genetic cause of death in infants. Their ability to correct a messenger RNA (mRNA) splicing defect in SMA that makes a gene called *SMN2* only partially functional forms the basis of a potentially powerful therapeutic approach. This year, Krainer and colleagues demonstrated that PTK-SMA1, a tetracycline-

like small molecule, stimulates production of SMN protein by directly targeting the splicing reaction in mouse models of SMA and in cells isolated from SMA patients. Further collaborative research will focus on preclinical drug development. Krainer's team has also uncovered important new details about how a mutation in a gene called *RARS2* causes a cellular editing error that results in a devastating brain disease called pontocerebellar hypoplasia (PCH).

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 and dynamics of nuclear polycomb complex proteins, known to keep genes in a silent state. The team seeks to target them to segments of DNA as a means of selectively silencing specific genes. Another focus is the study of long noncoding RNAs (ncRNAs) retained in the nucleus, whose functions are still mostly unknown. This year, Spector and colleagues revealed a unique structure-building role for two ncRNA molecules. Called MENε and MENβ, they organize and maintain the structure of paraspeckles, a compartment within the cell's nucleus.

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 when 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 origin recognition complex (ORC). Stillman's research has demonstrated that ORC is also involved in the process of segregating the duplicated chromosomes in mitosis. The team has found ORC at centrosomes and centromeres, structures that orchestrate chromosome separation in mitosis. This past year, they identified a protein, Orc1, that controls the copying of the centrosome in human cells and prevents it from being reduplicated. Orc1, which is one of six proteins that comprise the ORC, was also found by the lab to control the number of centriole pairs in a cell.

How is the very first step in the life of a protein functionally related to proteolysis, the very last step—the protein's destruction by the proteosome? The key link, William Tansey has 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 other components of the ubiquitin–proteasome system affect transcription. Recently, among other things, they have discovered that the proteasome itself interacts—likely in its entirety—with active genes, using its proteolytic functions to promote multiple steps in the transcription process. They are currently exploring the precise mechanism through which the proteasome is involved in gene activation and probing how this large protein complex is recruited to chromatin.

ORGANIZATION AND REGULATION OF TRANSCRIPTOMES

T.R. Gingeras P. Batut, K. Bell, S. Chakrabortty, C. Davis, A. Dobin, J. Drenkow, D. Fagegaltier, S. Jha, I. Lasa, W. Lin, J. Schlesinger, L.-H. See, H. Wang, C. Xue, C. Zaleski

ENCODE Project

C. Davis, A. Dobin, J. Drenkow, W. Lin, J. Schlesinger, H. Wang , C. Xue, C. Zaleski

A sizable fraction of our group is involved in the NHGRI-sponsored encyclopedia of DNA elements (ENCODE) project. Our group leads a consortium of five other international laboratories to identify and characterize the human transcriptome. Characterization of the transcriptome is divided into four parts. Long (>200 nucleotides) and short (<200 nucleotides) RNAs are being identified and characterized by our group and the Hannon group at CSHL, respectively. The 5′ transcription start sites (TSS) for nonpolyadenylated (poly(A)$^-$) and the 5′ and 3′ termini for polyadenylated (poly(A)$^+$) RNAs are being characterized by groups at RIKEN, Japan, and GIS, Singapore, respectively. Finally, cross-data-set integration analyses and full-length isolation and sequencing of novel RNAs are being addressed by CRG, Spain, and the Universities of Geneva and Lausanne, respectively.

Sites of transcription have been mapped using both high-density tiling arrays and deep RNA sequencing. The production of genome-wide transcriptome maps by the consortium has exceeded what was originally proposed as milestones for the first 2 years of the project. A total of 66 data sets have been submitted for the scientific community to use as a collective resource. The data sets that have been submitted are summarized in Table 1.

The sources of the long and short RNAs mapped in these experiments include eight primary and transformed cell lines. Long and short RNAs for all cell lines have been independently isolated from the nuclei and cytosol of each cell line. Additionally, for K562 cell lines, four other subcellular compartments have been analyzed for the RNA complement present. These compartments include nuclear chromatin, nucleoplasm and nucleoli, and cytosolic polysomes. Cross-subcompartment analyses of the maps are currently in progress.

ModENCODE Project

C. Davis, A. Dobin, D. Fagegaltier, S. Jha, C. Xue

A second transcriptome mapping project is also under way in our group. The NHGRI-sponsored model genome ENCODE (modENCODE) project is focused on defining the transcriptome mapping of two model genetic organisms: *Drosophila melanogaster* and *Caenorhabditis elegans.* Our group is one of seven laboratories that form a consortium seeking to identify and characterize the transcriptome of *D. melanogaster*. Our aim is to generate a comprehensive set of developmentally staged and tissue- and cell-specific RNAs for expression profiling using high-density genome tiling microarrays and deep RNA sequencing. As with the ENCODE project, these maps include long and short RNAs and sequences derived from the 5′ TSS.

Table 1. Summary of Production for Years 2001 and 2002

Group	Map type or output	Year 2001		Year 2002		Total submitted/ proposed
		proposed	submitted	proposed	submitted	
Gingeras	long RNAs	6	11	12	17	30/18
Hannon	short RNAs	3	3	6	6	9/9
	RIP/CLIP	3	0	3	0	0/6
Carninci-RIKEN	CAGE	3	2	6	9	11/9
Ruan-GIS	PET	3	1	6	15	16/9

In the current year of this project, we generated 3.5 billion 75-bp reads (single and paired ends) from 30 developmental time points using the Illumina GAIIx platform. Alignment of the data to combined genome and splice junction databases yielded ~2 billion uniquely mapped sequence reads. These data have been submitted for use by the scientific community and the underlying data sets have been submitted to GEO (Gene Expression Omnibus) and Short Read Archive. Analysis of the ~3.5 billion RNA-Seq reads from the developmental time course has identified ~29,000 unannotated splice junctions. Many of these junctions are supported by expressed sequence tag (EST) or cDNA sequences that are not incorporated into the current annotations. Moreover, 1746 of these junctions have been targeted for validation by directed experiments (see below). The paired-end RNA-Seq data provide important connectivity information, in some cases confirming up to four exons in a single read-pair.

FUNCTIONAL SHORT RNAS

Identification of Two Types of Short RNAs at the 3′ Ends of Genes

D. Fagegaltier, A. Dobin, C. Xue

Early in 2009, we reported a new posttranscriptional processing pathway leading to cleaved and capped long RNAs (>200 nucleotides) and capped small RNAs (<200 nucleotides). The capped short RNAs include those found at the 5′ and 3′ ends of most protein-coding and noncoding genes, namely, promoter-associated small RNAs (PASRs) and termination-associated small RNAs (TASRs) (Fejes-Toth et al. 2009). A third class of capped small RNAs spans across internal exons. Initially identified in humans and mice, it is now clear that such small RNAs derived from processed transcripts also exist in flies. However, their exact role in *Drosophila* transcription regulation is yet to be demonstrated.

One of the modENCODE consortium goals is to profile all capped small RNAs expressed in flies. To accomplish this goal, the development of a working protocol to clone enriched populations of capped small RNAs (<200 nucleotides) was required. Using A-tailing and anchored primers for reverse transcription, libraries were made and sequenced from S2 cell capped small RNAs, mixed capped and uncapped small RNAs, and 5′-monophosphate small RNAs. Sequences were mapped using Galaxy/Nexalign and the STAR algorithm developed in the laboratory (see below). As expected, microRNAs (miRNAs) are cloned in monophosphate libraries and absent from capped libraries; in turn, species known to be capped such as small nuclear RNAs (snRNAs), are mostly restricted to capped libraries. The analysis of unique mappers from the respective libraries also reveals several populations of both known and unknown classes of small RNAs: (1) Abundant capped small RNAs such as PASRs (Fig. 1, panel 1) and TASRs (Fig. 1, panel 2) present the same features as in human cell lines; one advantage of the protocol developed is that it is designed for paired-end stranded sequencing, allowing us to examine the size of these RNA classes. (2) New species of capped small RNAs are found at the 5′ and 3′ ends of specific families of transposable elements, whereas characterized somatic endoribonuclease-prepared siRNAs (esiRNAs) (5′-mono-P small RNAs) cover the internal regions of transposable elements. (3) Another interesting population of small RNAs emerged as 5′-mono-P small RNAs whose 3′ end aligns with the transcription termination (Fig. 1, panel 4; not present in panel 3).

Role of Short RNAs in Dosage Compensation

D. Fagegaltier

Our analysis of short RNAs in *Drosophila* has also prompted us to investigate the role of short RNA in sex differentiation. The X chromosome carries hundreds of genes, but few have anything to do directly with gene-specific expression. Thus, to function in both sexes, X-linked genes must be expressed at similar levels from a single copy in the heterogametic sex or two copies in the homogametic sex. To solve this conundrum, males (XY) and females (XX) equalize the level of X-linked transcripts through a process called dosage compensation. In *Drosophila*, at least six proteins (MSL-1, MSL-2, MSL-3, MLE, MOF, JIL-1) and two long noncoding RNAs (*roX1* and *roX2*) form a ribonucleoprotein complex whose association at hundreds of sites along the X chromosome exclusively in males enables doubling of the expression of X-linked genes. Hence, males generate from a single X chromosome transcripts equal in amount to their female counterparts. In females, the key player in sex determination, *Sex-lethal* (*Sxl*), represses MSL-2 expression, therefore preventing dosage compensation.

In flies, miRNAs are likely candidates for controlling the expression of genes involved in dosage compensation and in sex determination. Many of the annotated genes involved in these processes represent predicted targets of miRNAs in databases, but they require further in vivo validation. Eight to 27 predicted miRNA target sites are

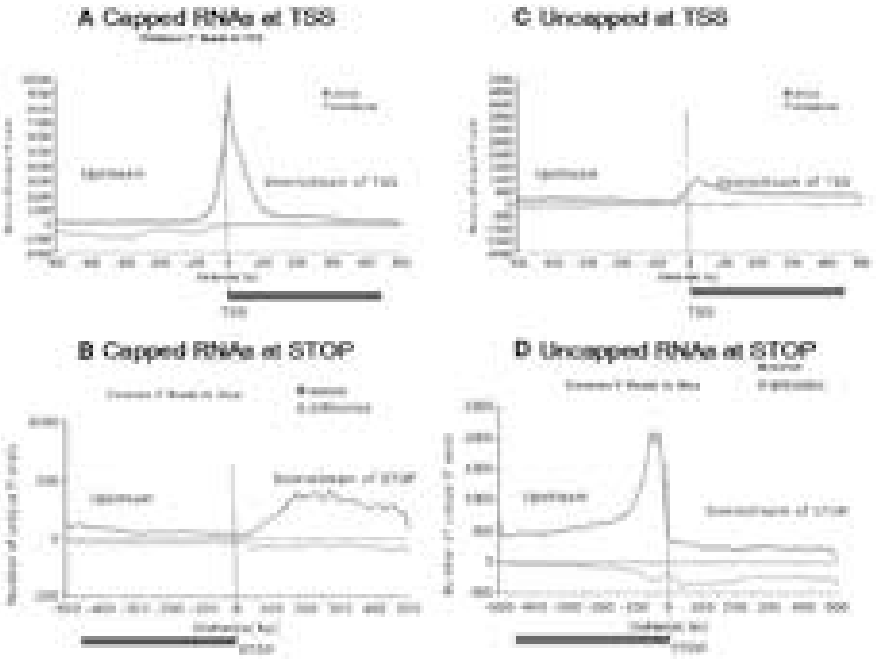


Figure 1. Density plots of RNASeq reads. (*A*) The capped short (s) RNAs found at the 5′ ends of fly genes are centered at the TSS of the gene and appear both on sense strands (above reference lane) and on antisense strands (below reference lane). (*B*) Capped sRNAs at the TSS are observed after the termination of the gene. (*C*) Uncapped sRNAs that are 10 times less exist at the TSS and (*D*) uncapped sRNAs map within the gene boundary before the TSS.

found in the 3′ untranslated region (3′UTR) of dosage compensation complex (DCC) components. MSL-3 is the only exception, as the predicted target of three miRNAs. Interestingly, the analysis of predicted miRNA targets points to four components of the DCC being targeted by a common miRNA (miR-985). The same applies to components of the sex determination hierarchy, whose 3′UTR contains 10–29 predicted miRNA target sites: miR-1000 has predicted target sites in *dsx, fru, tra*, and *sxl* 3′UTRs. In addition, two of these genes are predicted targets of the same four miRNAs.

One simple way of achieving sex-specific expression is to restrict expression of their miRNA repressors to the opposite sex. Recent studies in worms have identified miRNAs with sex-preferential expression. To determine the levels of expression of miRNAs predicted to target DCC and sex determination components, the populations of small RNAs in *Drosophila* male and female somatic tissues, respectively, have been surveyed. These include larval salivary glands, young adult heads and bodies, and somatic S2 cells derived from late embryos, an established male model for dosage compensation studies. Comparing the relative miRNA populations in sexed tissues using high-throughput sequencing revealed miRNA differentially or exclusively expressed in one sex. The widest set of miRNAs (all annotated 155 miRNAs) is detected in the fly body. More than 38 miRNAs show a strong bias toward male expression in body for 51 preferentially expressed in female (24 strongly biased). In salivary glands, 57 miRNAs present at least 50 reads across male and female libraries; ~110 miRNAs are detected in salivary glands when adding the numerous miRNAs with very few reads. Sixty are enriched preferentially in males, versus 47 in females. In heads, 71 miRNAs are preferentially expressed in males and 63 are preferentially expressed in females, out of 148 miRNAs detected; of these, two of the most abundant miRNAs show a strong male preference, and four abundant miRNAs show a female preference. In addition to sex biases, some miRNAs appear to be strongly enriched in specific tissues. Heads present a high number of expressed miRNAs, some of which are strongly enriched compared to young adult bodies. Fewer miRNAs are found in salivary glands compared to body samples covering a complex range of tissue types.

An important finding is that miRNAs expressed in bodies whose expression is the most strongly biased toward male are enriched on the X chromosome (13 of 38). Although the number of miRNAs found on the X chromosome is comparable to other chromosomes, dosage

compensation may provide a means of tuning miRNA expression on the X chromosome. A lower yet significant proportion of miRNAs whose expression is biased toward female is also found on the X chromosome (6 of 24). miRNAs found at similar levels in male and female bodies tend to be located elsewhere in the genome.

DEVELOPMENT OF ANALYSIS ALGORITHMS AND DATABASE

Development of RNA Mapping Algorithm

A. Dobin

The recent advances in sequencing technology made it an attractive tool for the studies of the transcriptome at single-nucleotide resolutions. The analysis of the tens of millions of relatively short (36-mer) and medium (100-mer) reads produced by sequencing of cellular transcriptomes comprising spliced and unspliced RNAs presents unique challenges. Two main tasks make these analyses extremely computationally intensive: The first task is the accurate alignment of increasingly longer reads that display growing numbers of mismatches caused by sequencing errors, single-nucleotide polymorphisms (SNPs), RNA editing, etc. The second task is the mapping of reads derived from noncontiguous regions of the genome, for example, splice junctions and chimeric RNAs. Although the first task is shared with DNA resequencing efforts, the second task is specific and crucial to the RNA sequencing because it provides the connectivity information and allows the reconstruction of the RNA molecules.

Various sequence alignment algorithms have been applied to these problems, including existing general-purpose approaches (e.g., BLAT) or more recent tools designed for high-throughput sequencing reads (e.g., Bowtie/Tophat). The latter strategies rely upon either previously annotated splice junctions, the sequence characteristics of annotated junctions (e.g., canonical introns motifs and maximum exon/intron size), and/or the construction of a reference database of junction sites. We use the splice transcripts alignment and reconstruction (STAR) tool. Its novel alignment strategy for de novo detection of the splice junctions does not require any previous knowledge of splice junctions' loci and does not use a priori properties of the junctions. This unbiased splice junction mapping is imperative for discovery of novel (unannotated, noncanonical) splice junctions and isoforms, as well as other increasingly important RNA species such as interchromosomal chimeric RNAs. The key algorithm of our alignment strategy is the search for the *maximum mappable length* of a read, implemented as a speed-efficient suffix array search. Another important novelty of our approach is the split/search/extend algorithm driven by the sequencing quality scores, which allows a confident alignment of reads comprising a large number of sequencing errors. The STAR's penalty system is highly user-configurable and assigns probabilistic meaning to the alignment scores.

The following are some other important advantages of the STAR: STAR is capable of finding the multiple loci to which a read can be mapped, providing estimates of the relative probabilities of these alignments. STAR can align reads of any length, working accurately and efficiently for both long and short RNA molecules. STAR can align reads containing any number of splice junctions, indels, and/or mismatches, which is important as the length of the reads continues to increase rapidly with the advances of the sequencing technology. STAR can deal with arbitrarily large intron length (important, e.g., for distal exons and chimeric RNA), as well as with extremely short exons (microexons). STAR performs an "auto"-trimming of the poor-quality read ends, which are a common occurrence as the read length is pushed to the limit. STAR can detect the nontemplated poly(A) tails, thus providing a means to determine the transcription termination site for poly(A^+) mRNAs. STAR treats the paired-end sequencing in a most straightforward way by incorporating naturally the paired-end information into the mapping process.

Although the STAR algorithm is heuristic and nonexhaustive (i.e., it does not find *all* the possible alignments, unlike, e.g., the Smith–Waterman local search), we showed that it can recover almost all highly probable alignments for almost all reads. STAR is very fast: On a modern but not overly expensive server, it can align 75 million per hour of 76-mer reads to the human genome, including the splice junctions, multiple mappers, and numerous (about seven) mismatches.

In the following example, we use STAR to map 270M 76-mer reads from a human cell line (K562) total RNA samples. Table 2 compares STAR's results with those of an exhaustive mapping with up to two mismatches allowed. One can see that for these libraries, STAR maps twice as many reads as the full-length (76 nucleotides) exhaustive search, or 50% more reads than the exhaustive search with the reads trimmed to 50 nucleotides, illustrating STAR's ability to autotrim the poor-quality tails.

We found 3.75M reads that cross splice junctions, from those 96% crossing canonical junctions (GT/AG

Table 2. STAR Performance Compared to Exhaustive Mapping Approach

	Uniquely mapped reads	Mean mapped length (bases)
0–2MM[a]	51M[b]	76
0–2MM & trim to 50	72M	50
STAR	106M	64.8

[a]Mismatch.
[b]Million.

introns) and 4% noncanonical. We found 87K unique junctions in the genome, of which 90% are annotated canonical junctions, 7% unannotated canonical, and 3% noncanonical mostly unannotated junctions. This illustrates STAR's ability to correctly detect annotated junctions, as well as predict a large number of unannotated canonical and noncanonical sites.

Novel Exon Detection by Hierarchical RNA-Seq Clustering

F. Schlesinger

Large-scale RNA sequencing projects, such as those done as part of the ENCODE project, allow the discovery of previously unknown transcripts or novel exons within known transcripts. Millions of short sequence reads derived from cDNA are independently mapped to the genome. Highly expressed regions are fully covered with reads, so that transcripts can easily be detected. However, many biologically relevant transcripts are only expressed at low levels of a few molecules per cell. Shotgun cDNA libraries are unlikely to completely cover such transcripts given current limitations to sequencing depth. Instead, the observed reads must be treated as a statistical sample of a much larger true population of RNA fragments.

Exons in low-abundance transcripts can still be seen as several reads mapping closer together in genomic space than would be expected by pure chance if they were evenly dispersed through the genome. To detect loci where such significant read clusters are observed and to distinguish them from nonspecific background events, we developed software to apply a hierarchical clustering algorithm based on a statistical sampling model and maximum likelihood model comparison.

This algorithm scans along a chromosome to find a large region with a higher density of mapped reads than the local background level. This region is then recursively split again into subregions according to read density until all such clusters are found. The benefit of this approach is that clusters of any length or strength can be detected independent of any predefined cutoffs while considering nonspecific background noise. This is important because we do not know a priori how long the novel transcripts of unknown type (spliced/unspliced, A^+/A^-) will be.

The largest clusters represent gene islands, whereas the lowest level detects individual exons. These blocks can be linked to form transcript models by split reads. These are short sequences derived from spliced transcripts, which span an exon–exon boundary. Detecting them at novel, possibly noncanonical splice sites requires the new STAR genome mapping software.

Results of this approach on total cellular RNA libraries show many novel transcribed loci, antisense transcripts, or extended 3′UTRs in agreement with previous tilling array analysis. RNA data from a single cell line sample included 20,000 intergenic clusters and more than 15,000 in antisense to genes, representing potential exons of novel transcripts present at levels corresponding to ~1.5 molecules per cell on average. We also observed more than 80,000 cases of RNA-Seq signal in introns of annotated transcripts. Further statistical analysis of their distribution and split-read mapping will help differentiate unspliced pre-mRNAs found in the total RNA library from novel exons.

Development of Genomic Data Manager Database and Pipeline

C. Zaleski

Next-generation genomics technologies such as microarrays and high-throughput sequencing are becoming more common among biologists. Although these technologies are extremely powerful, the information they generate introduces many difficulties. Among these are (1) analysis of the data, often requiring complex algorithms and tremendous computing resources, (2) visualization of the data, requiring powerful application interfaces and time-consuming study, and (3) storage and management of the data, requiring nontraditional approaches due to the size and complexity of the data.

Although many tools have addressed the first two points—analysis and visualization—we feel that storage and management of the data are an equally important, yet overlooked, part of the process. Traditional methods for handling this requirement could include files organized in a directory structure on a network drive, shared spreadsheets, and simple database solutions such as Access or Filemaker. However, these solutions often do not scale

to the needs of next-generation technologies due to

- *Number of files:* Easily reaches tens or hundreds of thousands.
- Files sizes: Up to many gigabytes for a single file.
- *Metadata:* A tremendous amount of information needs to be associated with the data at many levels of organization.

To address these storage and management issues, we created Genomic Data Manager (GDM). GDM is a web-based database system designed to solve the problems specific to storage and management of genomics data. It allows for the following:

- *Robust storage:* Data files are kept on a storage system that provides high-speed access, redundancy, and managed backups.
- *Organization and structure:* Experiment results (files) are organized into logical groups (aka "DataSets") that form hierarchical relationships. Each DataSet has an associated "Process" that created it.
- *Extensive metadata:* Detailed information can be associated with the data at multiple levels—Processes, DataSets, and Files—through the use of predefined attributes and user-defined annotation.
- *Security:* Multiple levels of access rights are enforced, and users can only view or manipulate data for the particular "projects" with which they are associated.
- *Easy search and retrieval:* Users can browse through data using a top-down hierarchy of biological attributes (e.g., species, cell line, and nucleic acid). Search features are also provided to quickly target a DataSet of interest. Requested data can be viewed simultaneously with all associated information, and data files can be downloaded with a single click.
- *Easy integration with analysis tools:* Although analysis of data is left to other existing tools, easy integration with these tools is vital. GDM has been integrated with a Galaxy (http://galaxy.psu.edu/) server and allows for seamless import/export while maintaining a homogeneous interface. Similarly, Galaxy itself can then be used to easily integrate almost any analysis tool that is required.

Integration with Galaxy is accomplished by using Galaxy's built-in facilities for external data sources. The Galaxy interface is divided into frames or sections that provide different information to the user—Menu, History, etc. When the user chooses to import data from an external source, the actual website of the source can be placed directly inside the Galaxy window. This is exactly how GDM works, and it allows for a seamless experience for the user. Once GDM was made aware of Galaxy and the appropriate configuration files were created, adding the GDM "tool" as a Galaxy data source is a plug-and-play procedure.

GDM was created with common tools and technologies allowing for easy maintenance. The database is implemented in MySQL 5.1, business logic is implemented with PHP 5.1 served via Apache 2, and the interface is created with HTML and Javascript, using widgets and Ajax from dhtmlx (http://www.dhtmlx.com/).

PROKARYOTIC TRANSCRIPTOMES

Transcriptional Profiles of *Staphylococcus aureus*

I. Lasa, A. Dobin

Due to their small size (on average between 2 and 5 Mb), bacterial genomes were the first free-living organisms to be sequenced. Almost immediately, the development of microarray techniques combined with the genome information allowed the characterization of what at that time was called "whole bacterial transcriptome." However, those initial studies were limited to the characterization of the transcription products of annotated genes. During the past few years, the development of high-resolution tiling arrays and RNA deep sequencing has allowed the study of transcriptomes without bias from previous genome annotations. These techniques, first implemented for eukaryotic organisms, have demonstrated that the collection of RNA molecules produced by eukaryotic cells (transcriptome) is highly more complex than expected. Although prokaryotic cells do not posses the elaborate RNA maturation machinery of eukaryotic cells, it seems likely that the bacterial transcriptome is going to be also more complex than expected.

This project is aimed at determining the transcriptome map of the pathogenic bacteria *Staphylococcus aureus*, combining tiling arrays and deep-sequencing technologies. *S. aureus* is a human pathogen that causes both nosocomial and community-acquired infections that can range from minor skin infections to life-threatening illnesses such as endocarditis, osteomyelitis, and toxic shock syndrome. From the analysis of the *S. aureus* transcriptome, we expect to (1) identify putative new families of ncRNA, (2) determine the frequency of RNA regulatory mechanism such as 5′ and 3′ overlapping UTR, antisense RNAs, (3) identify enzymatic activities linked to new RNA regulatory mechanisms that might be susceptible for the design of new antibiotics, and (4)

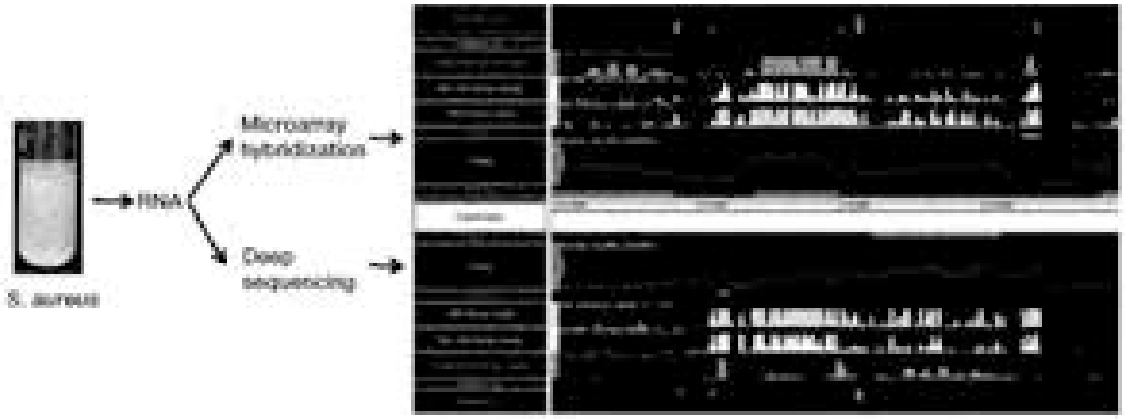


Figure 2. Array: The plots show the normalized hybridization intensities (*y* axis) and genome position (*x* axis). Each dot corresponds to the average intensity of intensity signals from independent biological repetitions.

define a new map of operons that will pave the path for all future genetic studies with this bacterium.

For tiling array studies, total RNA from three genetically unrelated *S. aureus* strains and selected isogenic mutants from one of these strains were hybridized with custom-made Affymetrix arrays. Analysis of the data was based in Bioconductor R package.

For RNA deep-sequencing, RNA was size-selected in two fractions, one containing RNA molecules shorter than 75 nucleotides and the other one containing total RNA. Two libraries using standard laboratory protocols have been constructed and sequenced.

Overall, the results show a very good correlation on a gene-by-gene comparison between the deep sequencing and the tiling arrays. Figure 2 shows 5′ and 3′ overlapping UTRs of convergent genes or noncoding RNA (ncRNA).

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STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

L. Joshua-Tor C. Ashar, J. Calarco, E. Elkayam, C. Faehnle, S. Goldsmith, C. Kuhn, P.R. Kumar, C. Kuscu, T. Lavy, S.-J. Lee, S. Lin, T. Schalch, A. Tocilj

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 with 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, R.A. Martienssen, Cold Spring Harbor Laboratory, and 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 that use these pathways, but RNAi became an extraordinary useful and simple tool for gene silencing. Almost from its beginnings, people 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 work 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 nucleotides) 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 RNAs (siRNAs) or microRNAs (miRNAs)—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.

In the fission yeast *Schizosaccharomyces pombe*, assembly of centrometic heterochromatin requires the RITS complex, which consists of Ago1, Tas3, Chp1, and siRNAs derived from centromeric repeats. In the past year, we have shown from our crystal structure of Chp1's chromodomain in complex with a trimethylated lysine-9 H3 peptide (H3K9me) extensive sites of contact that contribute to Chp1's high-affinity binding (Fig. 1). We found that this high-affinity binding is critical for the efficient establishment of centromeric heterochromatin, but preassembled heterochromatin can be maintained when Chp1's affinity for H3K9me is greatly reduced.

The Different Faces of E1: A Replicative Hexameric Helicase

E.J. Enemark (now at St. Jude Children's Research Hospital, Memphis, TN), S.-J. Lee

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

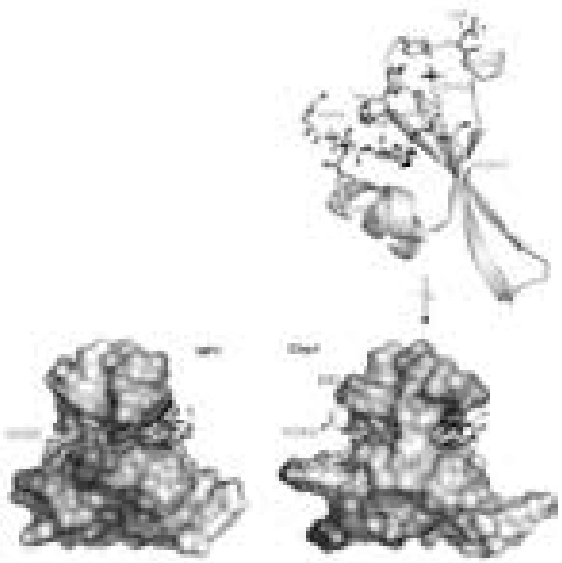

Figure 1. Crystal structure of the Chp1 chromodomain bound to H3K9me3 peptide. (*Top*) Cartoon representation of the Chp1 chromodomain is shown in a ribbon representation, and the H3 peptide is shown in a stick representation. The Chp1 "aromatic cage" residues and zinc-binding aspartates are also shown with sticks. Zinc ions are shown as gray spheres. (*Bottom*) Side-by-side comparison of HP1 and Chp1 cocrystal structures with H3K9me3 peptides. H3K9me3 peptide is shown with sticks.

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 papillomaviral 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, 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 while 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. A detailed comparison with other multimeric ATPase motors that highlighted the roles of individual site residues in the ATPase activity was also performed. We continue to study this helicase and the role other domains of the protein might have in helicase assembly and activity.

NADP Regulates the Yeast *GAL* Induction System

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

Transcriptional regulation of the 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 the dinucleotide NAD (nicotinamide adenine dinucleotide), 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.

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 was 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.

We are now examining possible enzymatic activities as well as other complexes involved in this classic transcriptional switch.

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RNA SPLICING

A.R. Krainer M. Akerman, O. Anczuków, I. Aznárez, C. Bennett, D. Chatterjee, S. Das, M. Faleiro, O. Fregoso, Y. Hua, M. Jensen, Y. Liu, Y.-H. Liu, L. Manche, H. Okunola, X. Roca, K. Sahashi, R. Sinha, S. Sun, R.-Y. Tzeng, M. Wallace, Z. Wang

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, especially in a disease context. A summary of some of our recently published studies is provided below.

Studies of Splicing Factors SF2/ASF and hnRNP A1

SF2/ASF is a prototypical serine- and arginine-rich protein, with important roles in splicing and other aspects of mRNA metabolism. *SFRS1*, the gene encoding SF2/ASF, is a potent proto-oncogene with abnormal expression in many tumors. We found that SF2/ASF negatively autoregulates its expression to maintain homeostatic levels. We characterized six alternatively spliced SF2/ASF mRNA isoforms: the major isoform encodes full-length protein, whereas the others are either retained in the nucleus or degraded by nonsense-mediated mRNA decay. Unproductive splicing accounts for only part of the autoregulation, which occurs primarily at the translational level. The effect is specific to SF2/ASF and requires RNA recognition motif 2 (RRM2). The ultraconserved 3′-untranslated region (UTR) is necessary and sufficient for down-regulation. SF2/ASF overexpression shifts the distribution of target mRNA toward monoribosomes, and translational repression is partly independent of Dicer and a 5′ cap. Thus, multiple posttranscriptional and translational mechanisms are involved in fine-tuning the expression of SF2/ASF.

Alternative splicing and posttranslational modifications (PTMs) are major sources of protein diversity in eukaryotic proteomes. Functional studies of SR protein PTMs have exclusively focused on reversible phosphorylation of serine residues in their carboxy-terminal RS domain. We confirmed that human SF2/ASF is methylated at residues R93, R97, and R109, which were first identified in a global proteomic analysis of arginine methylation. We further investigated whether these methylated residues regulate the properties of SF2/ASF, which normally shuttles between the nucleus and the cytoplasm. We found that the three arginines additively control the subcellular localization of SF2/ASF, and both the positive charge and the methylation state are important. Mutations that block methylation and re-

move the positive charge result in cytoplasmic accumulation of SF2/ASF. The consequent decrease in nuclear SF2/ASF levels prevents it from modulating alternative splicing of target genes, results in higher splicing-enhancer-dependent translation stimulation, and abrogates the enhancement of nonsense-mediated mRNA decay.

Heterogeneous nuclear RNP (hnRNP) A1 is a ubiquitous splicing repressor that binds to RNA in a cooperative manner. Initial hnRNP A1 binding to an exonic splicing silencer at the 3′ end of human immunodeficiency virus type 1 (HIV-1) tat exon 3, which is a high-affinity site, is followed by cooperative spreading in a 3′ to 5′ direction. As hnRNP A1 propagates toward the 5′ end of the exon, it antagonizes binding of SF2/ASF to an exonic splicing enhancer, thereby inhibiting splicing at that exon's alternative 3′ splice site. tat exon 3 and the preceding intron of HIV-1 pre-mRNA can fold into an elaborate RNA secondary structure in solution, which could potentially influence hnRNP A1 binding. We found that hnRNP A1 binding and splicing repression can occur on an unstructured RNA. Moreover, hnRNP A1 can effectively unwind an RNA hairpin upon binding, displacing a bound protein. We further showed that hnRNP A1 can also spread in a 5′ to 3′ direction, although when initial binding takes place in the middle of an RNA, spreading preferentially proceeds in a 3′ to 5′ direction. Finally, when two distant high-affinity sites are present on the same RNA, they facilitate cooperative spreading of hnRNP A1 between the two sites. These cooperative RNA-binding properties shed light into hnRNP A1's splicing repression activity, which controls alternative splicing of many pre-mRNAs.

Alternative Splicing and Cancer Cell Metabolism

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. In addition, alternative splicing controls a metabolic switch characteristic of all cancer cells, which is known as the Warburg effect. Cancer cells preferentially metabolize glucose by aerobic glycolysis, characterized by increased lactate production. This distinctive metabolism involves expression of the embryonic M2 isozyme of pyruvate kinase, in contrast to the M1 isozyme normally expressed in differentiated cells, and it confers a proliferative advantage to tumor cells. The M1 and M2 pyruvate kinase isozymes are expressed from a single gene through alternative splicing of a pair of mutually exclusive exons. In collaboration with Lewis Cantley (Harvard Medical School), we measured the expression of M1 and M2 mRNA and protein isoforms in mouse tissues, tumor cell lines, and during terminal differentiation of muscle cells and showed that alternative splicing regulation is sufficient to account for the levels of expressed protein isoforms. We further showed that the M1-specific exon is actively repressed in cancer cell lines—although some M1 mRNA is expressed in cell lines derived from brain tumors—and demonstrated that the related splicing repressors hnRNP A1 and A2, as well as the polypyrimidine-tract-binding protein PTB, contribute to this control. Down-regulation of these splicing repressors in cancer cell lines using short hairpin RNAs (shRNAs) rescued M1 isoform expression and decreased the extent of lactate production. These findings extended the links between alternative splicing and cancer and identified some of the factors responsible for the switch to aerobic glycolysis.

Spinal Muscular Atrophy: Genetics, Splicing Mechanisms, and Therapeutics Development

Spinal muscular atrophy (SMA) is a common, autosomal-recessive motor neuron disorder caused by homozygous deletion or mutation of the survival-of-motor-neuron gene, *SMN1*. A closely related *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. *SMN2* decreases the severity of SMA in a copy-number-dependent manner. However, *SMN2* is not the sole phenotypic modifier, because there are discordant SMA cases in which the *SMN2* copy number does not explain the clinical phenotype. In collaboration with Tom Prior (Ohio State University), we reported three unrelated SMA patients with low *SMN2* copy numbers that did not correlate with their mild clinical phenotypes. A single base substitution in *SMN2*, c.859G>C, was identified in exon 7 in the patients' DNA. We showed that the nucleotide change creates a putative exonic splicing enhancer motif and increases the amount of full-length transcript, thus resulting in the less severe phenotypes. This finding demonstrated that the c.859G>C substitution is a positive modifier of the

SMA phenotype and that *SMN2* sequence variations can affect the disease severity.

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 tetracycline derivatives, in collaboration with Paratek Pharmaceuticals. This work was initiated by Michelle Hastings, who is now at Roslind Franklin University, Chicago. We identified a tetracycline-like compound, PTK-SMA1, which stimulates exon-7 splicing and increases SMN protein levels in SMA patient fibroblasts and in mice transgenic for human *SMN2*. Unlike previously identified small molecules that stimulate SMN production via *SMN2* promoter activation or undefined mechanisms, PTK-SMA1 is a unique therapeutic candidate in that it acts by directly stimulating splicing of exon 7, as shown by in vitro splicing experiments in cell-free extracts. Structure-activity relationship studies are in progress to develop more potent derivatives, as well as compounds that efficiently penetrate the blood-brain barrier.

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. 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*, associated with a 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 one. 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.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector M.S. Bodnar, M. Eckersley-Maslin, S. Hearn, M. Hübner, R.I. Kumaran, Z. Lazar, J. Li, Y. Mao, H. Sunwoo, B. Zhang, R. Zhao

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 dynamics of a long nuclear-retained noncoding RNA.

The Dynamics of Gene Expression/ Repression and DNA Repair

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

Our research during the past year has focused on the spatial and temporal aspects of gene expression. During the cell cycle, development, and differentiation, the gene expression program of cells changes. These changes in gene expression are carried out in part by changes in chromatin-associated histone modifications. The JMJD3/KDM6B protein has recently been identified to act as a demethylase for dimethylated and trimethylated H3K27. Its action would thereby have a role in activating a gene that was silenced by the H3K27 trimethyl mark. During the past year, we have been interested in studying the nuclear organization and dynamics of the JMJD3 protein. In addition to a diffuse nuclear pool, endogenous JMJD3 protein detected by a specific antibody, or in a stable cell line expressing yellow fluorescence protein (YFP)-tagged JMJD3, localized to 20–40 nuclear foci that are 0.2–0.5 m in diameter in human U2OS osteosarcoma cells. These foci are relatively static structures and do not change their position relative to each other over several hours in interphase cells. The foci break up in prophase of mitosis and form once again in the G_1 phase of the cell cycle. Deletion analysis has shown that the nuclear localization of the JMJD3 protein requires the amino-terminal portion of the protein, whereas localization to nuclear foci depends on an intact zinc finger domain close to the carboxyl terminus. Conversely, the catalytic JmjC domain, or an intact Fe(II)-binding site in the catalytic domain, is dispensable for localization to JMJD3 foci. An examination of the dynamics of the JMJD3 protein by fluorescence recovery after photobleaching (FRAP) has shown that the population of the JMJD3 protein residing in foci has a relatively slow exchange rate, with a half-recovery time of 12 sec (+/– 1.9) and an immobile fraction of 20%. In contrast, the diffuse pool of JMJD3 has a fast half-recovery time of ~1 sec and no immobile fraction. Interestingly, the slow recovery of JMJD3 within foci depends on the presence of its catalytic domain, and removal of this domain leads to faster recovery (5.2 sec +/– 0.8) and the loss of the immobile fraction. These data suggest that it is the functional pool of the protein that is localized to JMJD3 foci. The JMJD3 foci do not show significant colocalization with sites of ongoing transcription (RNA polymerase II Ser-2-P, H3K4me3, AcH3). However, they colocalize with marks of heterochromatin (HP1α, HP1γ, H3K9me3). Similarly, the JMJD3 protein shows a low level of colocalization with a 200-copy transgene array stably integrated on human chromosome 1p36 in U2OS 2-6-3 cells (Janicki et al., *Cell 116:* 683–698 [2004]). Importantly, upon transcriptional induction of this locus by doxycycline, JMJD3 becomes rapidly (within 3 min) and transiently recruited to the locus. This recruitment precedes the detection of RNA transcripts by ~10 min, indicating that JMJD3 might have a role in the early events of transcriptional induction. In accordance with the live cell imaging data, chromatin immunoprecipitation experiments revealed that JMJD3 shows a low level of binding to the uninduced locus. Importantly, upon transcriptional induction, JMJD3 becomes recruited primarily to the boundary between the promoter and the coding region where it shows a threefold enrichment compared to the

uninduced state within 1 h of induction. In comparison, recruitment to the body of the coding region is slightly less (2.5-fold) and is weak within the 3′ untranslated region (3′UTR; 1.5-fold). Upon prolonged induction, JMJD3 levels at the locus decline, and after 4 h of induction, JMJD3 binding to the locus is twofold below the initial level of the uninduced locus. Next, we examined a second cell type to determine whether JMJD3 shows a localization to nuclear foci similar to that observed in U2OS cells and whether JMJD3 changes its localization upon transcriptional induction of endogenous genes by a ligand-activated stimulus. To this end, we used MCF7 human breast cancer cells, in which a number of well-characterized endogenous genes are induced by the steroid hormone estradiol. Similar to that observed in U2OS cells, JMJD3 localized to nuclear foci, albeit with a larger diffuse pool as compared to U2OS cells. Importantly, upon administration of 10 nM estradiol, these foci become more prominent transiently. Interestingly, upon short hairpin RNA (shRNA)-induced knockdown of JMJD3, the inducibility of estradiol-inducible genes is reduced, indicating that JMJD3 has a role in estrogen-receptor-mediated gene transcription. Experiments are under way to study the role of JMJD3 on estradiol-induced genes on a genome-wide level and to determine if estradiol-induced genes localize to JMJD3 foci in MCF7 cells.

A second major focus of our efforts during the past year has been a comparative study of the kinetics of transcriptional induction of the same genetic locus in interphase and postmitotic U2OS 2-6-3 cells. This cell system allows us to directly visualize gene expression (DNA, RNA, protein) within the context of a living cell. In interphase, after transcriptional induction, we observed decondensation of the locus from a heterochromatic 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 intensity of the locus over time, we found that RNA pol II levels and mRNA production gradually increased over time, 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 kept the locus in a transcriptionally active state and allowed 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 postmitosis, with mRNA production peaking at 15–30 min. This observation suggested that the cell is somehow able to remember, or "bookmark," the locus as one needing to be transcribed such that the transcription machinery can be rapidly recruited to the locus when cellular transcription is reactivated after mitosis. Interestingly, we detected a rapid recruitment of the chromatin remodeling protein Brd4 in early G_1, which occurs ~2–4 min earlier than RNA pol II and positive transcription elongation factor b (P-TEFb). Next, we examined whether specific histone modifications were also associated with the loci during mitosis. Chromosome immunoprecipitation (ChIP) experiments showed about fourfold enrichment of histone H4 lysine-5 acetylation (H4K5Ac), a mark recognized by Brd4, at the locus upon induction in interphase. These data suggest that both Brd4 and the histone modification are part of a "bookmarking" complex at the locus. Interestingly, when transcription elongation is inhibited prior to mitosis by treating cells with 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) and then releasing them and allowing the cells to proceed through mitosis, RNA pol II is still rapidly recruited to the locus after mitosis, although mRNA synthesis occurs more slowly. These results imply that transcriptional initiation and transcriptional elongation are separately bookmarked. This study has provided a real-time view of interphase transcriptional induction and postmitotic reactivation at a single genetic locus and provides a system in which we will continue to study how gene expression patterns are transmitted to daughter cells postmitosis.

In addition to examining the dynamics of transcription, we have also been interested in examining the assembly of the DNA-repair nanomachinery at sites of DNA damage. Current methodologies, to specifically correct mutations both reproducibly and robustly at a given site in the genome of mammalian cells, are limited. This is because of the high frequency of nonhomologous end joining (NHEJ) compared to the homologous recombination (HR) mechanism. However, engineered chimeric zinc finger nucleases (ZFNs) that combine the nonspecific cleavage domain of the FokI restriction endonuclease, with custom-designed zinc finger domains, specific to unique DNA sequences, represent a very promising approach because inducing a DNA break by ZFNs increases the frequency of HR. To visualize the repair process at high resolution, and in living cells, we have used U2OS 2-6-3 cells and a microscope platform called OMX.

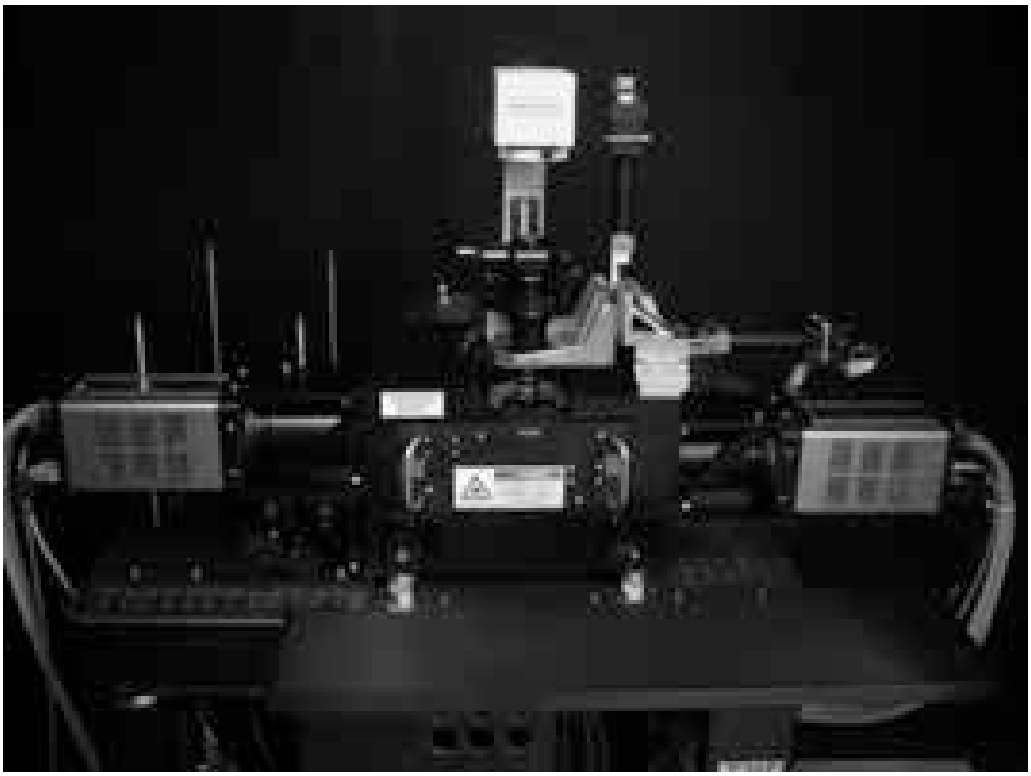

Figure 1. The OMX microscope is optimized for rapid live cell imaging and super-resolution (100 nm) imaging of fixed cells.

OMX (optical microscope experimental) is a new microscope platform (Fig. 1) with unprecedented mechanical and thermal stability, coupled with high sensitivity and increased signal:noise ratio for photon-limited bioimaging. We are currently one of seven laboratories in the world to have implemented this microscope system. In the structured illumination mode (SIM), OMX allows for imaging at super resolution of ~100 nm (beyond the diffraction limit). In the live cell imaging mode, the microscope allows for rapid image acquisition in four channels using EMCCD cameras. Thus, OMX is ideal for rapid/fast live cell imaging and super-resolution imaging. The OMX system is equipped with five laser lines (405, 457, 488, 514, and 594 nm). The twofold increase in resolution (X, Y, and Z) will provide a major advantage to our studies aimed at understanding the assembly of the DNA-repair machinary at the site of a single double-strand break (DSB) in our newly developed one-to-few copy DSB reporter cell lines. During the past year, we have optimized the three-dimensional (3D)-SIM imaging for the recruitment of the DNA-repair nanomachinary (mCherry-53BP1, EYFP-Rad51) to ZFN-induced DSB break sites marked by LacI-ECFP. Ongoing studies are aimed at elucidating the kinetics of assembly and disassembly of the DNA-repair machinery.

Identification and Characterization of Nuclear-retained Noncoding RNAs

Y. Mao, H. Sunwoo, B. Zhang

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 the 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 ncRNAs?

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 FISH (fluorescence in situ hybridization) analysis of candidate clones revealed one clone, corresponding to the MEN ε/β locus, that 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 MEN ε/β transcripts are localized to nuclear paraspeckles and directly interact with p54/nrb. Knock down 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 MENε/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.

In addition, during the past year, we have developed a stable mouse myoblast cell line in which we can inducibly express MEN ε RNA containing an MS2-conjugated fluorescent tag. Using this system, we have been able to demonstrate that paraspeckles are formed at the MEN ε transcription site. In addition, we have been able to characterize the dynamics of the MEN ε RNP with respect to its transcription site and other nuclear domains. We find several categories of dynamic events. In ~25% of the cells, the MEN ε RNPs form stable clusters, in 15% of the cells, they are highly dynamic, and in 60% of the cells, they are released from the transcription site and then move to other nuclear locations including existing paraspeckles. Ongoing studies are examining the function of MEN ε/β RNAs in differentiation.

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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. This is frequently the case, especially for cervical carcinoma. Indeed, HPV infection appears to be a necessary cause of invasive cervical carcinoma and thus represents one of the few firmly established links between viral infections and the development of cancer.

An impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for analysis of the viral life cycle. These viruses normally require specialized differentiating cells that only with difficulty can be generated in cell culture. However, for a bovine papillomavirus (BPV-1), a convenient cell culture system exists where viral gene expression, oncogenic transformation, and viral DNA replication can be studied. Thus, BPV has become a useful model for these aspects of the viral life cycle. The DNA replication properties of the papillomaviruses show some unique and interesting characteristics. As part of their normal life cycle, these viruses can exist in a state of latency, which is characterized by maintenance of the viral DNA as a multicopy plasmid in infected cells. The copy number of the viral DNA is tightly controlled, and the viral DNA is stably inherited under these conditions. Papillomaviruses therefore provide a unique opportunity to study plasmid replication in mammalian cells. In addition, the viral DNA replication (ori) 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 (ori) 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 10 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 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) forma-

tion, which require 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 formation. Interestingly, two of these mutants, D478A and N523A, showed significant trimer formation even in the absence of nucleotide. This result indicated that these two mutations might generate a conformational change that mimics the nucleotide-bound state. However, when we tested the mutants for DT formation in the absence of nucleotide, these mutants did not form DT but instead formed ladders. Upon addition of nucleotide, the DT was formed, demonstrating that these mutants are able to bind nucleotide. One interpretation of these results is that nucleotide binding serves two different functions for E1 DNA binding. The first function could be a general activation of DNA binding, which is mimicked by the mutants at residues 478 and 523. The second function could be a direct dependence on bound nucleotide for formation of the DT complex, which the mutations at 478 and 523 cannot mimic. The general lack of DNA-binding activity of E1 in the absence of nucleotide presents an obvious possibility. The E1 DNA-binding domain (DBD) may be regulated by the interaction with other parts of the E1 protein. To determine whether this was the case, we performed pull-down experiments using the E1 DBD and other parts of E1. Interestingly, the E1 DBD interacted strongly with a fragment containing the oligomerization and helicase domain fragments, and furthermore, this interaction was significantly weakened by the presence of nucleotide. These results indicate that the natural state of the E1 DBD in the absence of nucleotide is in a complex with the E1 helicase domain, where the DNA-binding surface of the E1 DBD is hidden. Upon addition of nucleotide, binding of the nucleotide to its binding site in the E1 helicase domain releases the E1 DBD from its interaction with the helicase domain and exposes the DNA-binding surface. These data for the first time provide a molecular explanation for how nucleotide binding activates DNA binding by replication initiator proteins.

Structure-based Mapping of the Nonspecific DNA-binding Activity in the E1 Helicase Domain

The E1 initiator protein contains at least two distinct DNA-binding activities. A sequence-specific DNA-binding activity, which we have characterized extensively, resides in the E1 DBD. This DNA-binding activity is required for tethering of the E1 protein to the viral origin of DNA replication, which contains four E1-binding sites (E1 BS). A poorly characterized DNA-binding activity is present in the helicase and oligomerization domain. This activity binds to double-stranded DNA without sequence specificity and is required for attachment of the E1 helicase domain to the sequences flanking the E1 BS to form an E1 DT complex on the ori DNA. The DT is required for local melting of the ori DNA and serves as a direct precursor for the E1 double hexamer, which is the replicative DNA helicase.

We have previously demonstrated that two residues (K506 and H507) in a β-hairpin structure present in the E1 helicase domain are required for this nonspecific DNA-binding activity. To further identify residues that might be involved in the nonsequence-specific DNA binding by the E1 helicase domain, we extracted a monomer of the E1 helicase and oligomerization domain from the hexameric structure and placed it on DNA such that the β-hairpin residues K506 and H507 were in contact with the DNA. On the basis of this model, we identified 14 residues in the helicase and oligomerization domains that might contact DNA. We mutated these residues to alanine and expressed and purified the mutant proteins from *Escherichia coli*. We screened the mutant proteins for E1 trimer and DT formation and identified three residues that in addition to the previously identified K506 and H507 affected the ability of E1 to form the E1 trimer and DT. These residues are present in three elements in the E1 helicase domain. The β-hairpin contains three residues (R505, K506, and H507) that are involved in nonspecific DNA binding. A hydrophobic loop contains F464 and in close proximity, a charged loop contains K461. To directly test whether these mutants affected the nonspecific DNA-binding activity of the E1, we used a competition assay where we challenged sequence-specific DNA binding of E1 to E1 BS with nonspecific competitor DNA. Due to the presence of the nonspecific DNA-binding activity in the helicase domain, binding of wild-type E1 is very sensitive to competitor DNA. Mutations that reduce the nonspecific DNA-binding activity show resistance to competitor DNA. The five mutants that were defective for trimer and DT formation all showed increased resistance to competitor DNA, indicating that these mutations specifically affected nonspecific DNA-binding activity. As expected, all of the mutations that affected nonspecific DNA binding were also defective for DNA replication in vitro and in vivo. These results identify a novel DNA-bind-

ing activity in the helicase domain of the E1 initiator proteins, which likely is conserved also for the T antigens of the polyomaviruses.

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Stephen Schuck

DNA REPLICATION AND CHROMATIN INHERITANCE

B. Stillman M. Hossain, N. Kara, H. Kawakami, J. Kinney, A. Mazurek, S. Mitelheiser, S. Ni, M. Rossmann, Y.-J. Sheu, K. Siddiqui, P. Wendel

Approximately 15 years ago, we successfully reconstituted the complete replication of the small simian virus 40 (SV40) genome with purified human cell proteins and the essential virus-encoded T antigen, which is the protein that serves multiple functions as an origin recognition protein, a DNA helicase, and a DNA primase and polymerase loading protein. That significant achievement allowed us to investigate how those proteins copied both strands of the double helix to produce two identical genomes and set the stage for understanding how cellular chromosomes duplicate. Just 2 years before this work was published, we reported the structure of the first eukaryotic cell origin of DNA replication to be characterized in detail and also identified the origin recognition complex (ORC), a six-subunit protein that binds to origins of DNA replication, thereby beginning the complex process of initiation of DNA replication throughout the entire cell genome. The discovery of ORC facilitated the rapid identification of other initiation proteins, such as Cdc6, Cdt1, and the MCM^{2-7} hexamer of proteins that combined to form a prereplicative complex (pre-RC) that renders chromosomes competent for subsequent initiation of DNA replication that occurs in S phase of the cell division cycle. Moreover, these studies resulted in a detailed description of how the initiation of DNA replication in eukaryotic cells is regulated throughout the cell division cycle and how DNA replication occurs only once before the execution mitosis. This year, we, in collaboration with former postdoctoral fellow Christian Speck, who now has his own laboratory at the MRC in London, reported the reconstitution of pre-RC assembly in vitro with purified proteins. John Diffely's laboratory at Clare Hall near London also reported in 2009 the reconstitution of pre-RC assembly in vitro with highly purified proteins. These two studies have now set the stage for understanding of how DNA replication forks are established in eukaryotic cells.

Mechanism of Pre-RC Assembly

A major goal of our studies on DNA replication was to identify the molecular mechanisms of pre-RC assembly, a process involving ORC, Cdc6, Cdt1, and the MCM^{2-7} proteins that are thought to be the essential core of the replicative helicase complex. The pre-RC is formed at each origin of DNA replication as cells exit mitosis or as cells progress though G_1 phase. Pre-RC assembly defines a state in which all chromosomes are licensed for a new round of initiation of DNA replication that occurs later in S phase, after cells commit to cell division. Thus, the molecular nature of the pre-RC and which proteins are both necessary and sufficient for its assembly have been of considerable interest. The ORC, Cdc6, Cdt1, and the MCM^{2-7} proteins were identified as essential for pre-RC assembly in vivo using a combination of genetics and biochemistry, but we did not know if they were sufficient. Other proteins, such as Noc3, have been implicated as essential for pre-RC assembly, but the role of these proteins has remained controversial.

In prior years, we have characterized the interactions between ORC and Cdc6 and have shown that Cdc6 binds to ORC and activates the ORC ATPase activity that it inhibited when ORC binds to origins of DNA replication. Furthermore, we have shown that addition of Cdc6 to ORC that is bound to the origins DNA greatly extends the nuclease protection footprint of the origin from 48 bp to more than 80 bp. The extended footprint of the ORC–Cdc6 complex requires the activity of the Cdc6 ATPase and specific nucleotides within the origin DNA. The assembly of the ORC–Cdc6 complex was thought to be a precursor for loading of the MCM^{2-7} complex onto DNA by the Cdt1 chaperone. In the past year or two, we have focused on the loading of the MCM^{2-7} complex.

A protein complex consisting of Cdt1 bound to MCM^{2-7} was purified from yeast cells that had been arrested in G_1 phase. This complex did not exist in S- or G_2-phase cells. We were able to express Cdt1 in *Escherichia coli*, and Christian Speck, when he moved to his own lab at the MRC in London, was able to overexpress and purify the MCM^{2-7} complex from G_1-phase yeast cells. When combined with ORC and Cdc6, the Cdt1 and MCM^{2-7} complex were active in loading the MCM^{2-7} proteins onto DNA in a highly efficient man-

ner. $MCM^{2–7}$ complex loading was dependent on ORC, Cdc6, and Cdt1, and in the presence of competitor DNA, the loading occurred specifically at the genetically defined origin of DNA replication. In the absence of competitor DNA, efficient $MCM^{2–7}$ complex loading was achieved on nonorigin DNA. Other proteins such as Noc3 were not required. The loading was ATP-hydrolysis dependent.

The $MCM^{2–7}$ complex purified from yeast cells was a hexamer consisting of one of each of the Mcm2, Mcm3, Mcm4, Mcm5, Mcm6, and Mcm7 proteins; however, after loading, the $MCM^{2–7}$ proteins were now present in a double hexamer (dodecamer) structure. Moreover, the dodecamer of $MCM^{2–7}$ was able to slide along the DNA because it was stably associated with circular DNA, but slid off linear DNA. When the linear DNA was blocked with a streptavidin protein bound to a biotinylated nucleotide at the ends of the linear DNA, sliding off the DNA was prevented. We concluded that the $MCM^{2–7}$ dodecamer complex was topologically linked to the double-stranded DNA and that it could slide, but dissociate from, the double-stranded DNA unless a double-strand break occurred in the DNA.

These results were very similar to results published at about the same time from John Diffley's laboratory (Remus et al., *Cell 139:* 719 [2009]), and combined, they represent a major advance in understanding the mechanism of initiation of DNA replication. We now know that for loading of $MCM^{2–7}$ complex, ORC, Cdc6, and Cdt1 are sufficient, but the results raise many interesting questions, particularly how is a double hexamer of $MCM^{2–7}$ loaded onto DNA by an apparent single ORC and Cdc6 complex and how can the double hexamer be activated to become a DNA helicase? We are investigating these and other questions.

To facilitate the biochemical characterization of pre-RC assembly and subsequent steps in the initiation of DNA replication, we have expressed the $MCM^{2–7}$ proteins in baculovirus-infected insect cells and have purified the $MCM^{2–7}$ complex using Cdt1 protein affinity chromatography. In this manner, a highly purified Cdt1–$MCM^{2–7}$ complex can be obtained, and it also supports pre-RC assembly in vitro with purified ORC and Cdc6.

Control of the Initiation of DNA Replication by the Kinase DDK

In the last year, we have reported that the major and essential target of the Cdc7-Dbf4 (DDK) protein kinase is the amino terminus of the Mcm4 protein. We also demonstrated that the Mcm4 amino terminus contains both inhibitory and activating regions for the initiation of DNA replication after formation of the pre-RC, a process that requires both DDK and the S-phase-specific cyclin-dependent protein kinase CDK. We reported that deletion of the inhibitory sequences from the Mcm4 amino terminus was sufficient to bypass the dependence on DDK for cell viability and initiation of DNA replication. This argued that the Mcm4 amino terminus was the critical and essential target for regulation by DDK, but it did not mean that this was the only target.

In an effort to identify other DDK targets, we collaborated with Mike Snyder and Ben Turk at Yale who had devised a high-throughput method for analysis of large number of peptides that each had a carboxy-terminal biotin tag as substrates for various protein kinases. The phosphorylated peptides were identified by printing the peptides on a streptavidin-coated array and detecting the phosphorylation. Using this method, they applied DDK along with 60 other protein kinases from yeast, and based on the peptide sequence, the DDK was found to have a remarkably relaxed requirement for amino acid sequence at the site of phosphorylation. The study linked DDK to the acidophilic protein kinases that included Polo-like kinase (Cdc5), casein kinases 1 and 2 (CK1 and CK2), and glycogen synthase kinase 3 (GSK3). Thus, the specificity of the kinase was not embedded in the substrate, but most likely how the kinase binds to its substrate. We have shown that DDK binds to a docking site in Mcm4 that is adjacent to the phosphorylation site. This opens the door to perform a screen for DDK-binding proteins as potential substrates.

Role of ORC in Human Cells in the Maintenance of Heterochromatin

The ORC is generally known as a DNA replication initiator protein, but during recent years, we and other investigators have shown that it is involved in diverse cellular functions including gene silencing, sister-chromatid cohesion, telomere biology, heterochromatin localization, centromere and centrosome activity, and cytokinesis. Recent studies of ORC in human cells have revealed that multiple ORC subunits associate with HP1α and HP1β containing heterochromatic foci. By investigating the recovery of fluorescently labeled ORC subunits that were photobleached in vivo by lasers, we have shown that multiple subcomplexes

of ORC exist at heterochromatin, with Orc1 stably associated with heterochromatin in G_1 phase, and other ORC subunits having more transient interactions throughout the cell division cycle. Both Orc1 and Orc3 directly bound to HP1α and two domains of Orc3—a coiled coil domain and a MIR domain—can independently bind to HP1α; however, both are essential for in vivo localization of Orc3 to heterochromatic foci. Direct binding of both Orc1 and Orc3 to HP1 suggests that following the degradation of Orc1 at the G_1/S boundary, Orc3 facilitates assembly of ORC/HP1 proteins to chromatin. Although depletion of Orc2 and Orc3 subunits by small interfering (siRNA) caused loss of HP1α association to heterochromatin, loss of Orc1 and Orc5 caused aberrant HP1α distribution only to pericentric heterochromatin surrounding nucleoli. Depletion of HP1α from human cells also shows loss of Orc2 binding to heterochromatin, suggesting that ORC and HP1 proteins are mutually required for each other to bind to heterochromatin. Similar to HP1α-depleted cells, Orc2 and Orc3 siRNA-treated cells also show loss of compaction at satellite repeats, suggesting that ORC together with HP1 proteins may be involved in organizing higher-order chromatin structure.

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REGULATION OF TRANSCRIPTION BY THE UBIQUITIN–PROTEASOME SYSTEM IN NORMAL AND CANCER CELLS

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

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

Regulation of Myc by Ub-mediated Proteolysis

The c-*Myc* oncogene encodes a basic helix-loop-helix leucine zipper transcription factor that can act as both a transcriptional activator and a repressor. First identified as a cellular counterpart of the transforming gene from the avian myelocytomatosis virus MC29, Myc has been shown to have an extensive role in cancer: It can collaborate with other oncogenes to induce cellular transformation, and aberrant Myc expression is observed in many human malignancies, including adenocarcinoma, colon, breast, and lung cancer, as well as a variety of leukemias and lymphomas. Indeed, it has been estimated that one-third of all human cancers display deregulated Myc activity and that one American dies every 7 minutes as a result of ectopic Myc expression or activity.

The majority of evidence indicates that Myc exerts its biological effects by controlling transcription. Activation of Myc target genes depends on the association of Myc with its partner protein, Max, and is mediated via the actions of an amino-terminal transcriptional activation domain (TAD) that interacts with components of the transcriptional machinery. A large number (~1700) of Myc target genes have been identified, including an impressive assortment of cell-cycle regulators, metabolic enzymes, and components of the translational machinery. The ability of Myc to regulate so many genes important for cell proliferation and growth undoubtedly underlies its ability to promote ectopic S-phase entry, apoptosis, and cellular transformation.

Because of its potent growth-promoting properties, cells have evolved a number of mechanisms to limit Myc accumulation and activity. One of the most prominent of these mechanisms is Ub-mediated proteolysis, which typically destroys Myc within minutes of its synthesis. The rapid and controlled destruction of Myc keeps its levels low and has a major role in restraining Myc's function, a notion supported by the fact that tumor-derived mutations within Myc block its rapid destruction and are aggressively oncogenic. Our laboratory is interested in the mechanisms and consequences of Myc destruction by the Ub–proteasome system. During the years that we have studied this process, we have defined the elements in Myc that control its stability, identified proteins that interact with these elements, and probed how the turnover of Myc impacts its activity as an transcription factor and oncoprotein.

In the last year, we have pursued three main avenues of research on Myc. First, we have probed the role that a conserved region known as "Myc box III" (MbIII) has in the function of Myc. Our previous studies have revealed that this element is important for signaling Myc proteolysis and that in the absence of this conserved sequence, Myc becomes "hyperapoptotic" and hence less able to drive cellular transformation. In the last year, we identified a number of protein factors that interact with this region of Myc and are in the process of asking how these factors influence Myc stability and activity.

Second, we revisited the issue of whether the Ub-mediated proteolysis of Myc is subverted in cancer. Our work on this topic goes back to the early days of the laboratory, when we found that a commonly occurring tumor-derived mutation in Myc, threonine 58 to ala-

nine (T58A), blocks its rapid destruction by the ub–proteasome system. Subsequently, in collaboration with the laboratory of Dr. Scott Lowe at CSHL, we found that this mutation also enhanced the oncogenic potential of Myc and allowed Myc to drive tumorigenesis in vivo without triggering p53-dependent tumor surveillance mechanisms. Although we did not explore at the time the extent to which tumor-derived mutations affect Myc, we have addressed this topic in the last year. Analysis of the literature reveals that in ~50% of cases where the Myc gene is rearranged, there is some change to the Myc coding sequence. Interestingly, these changes cluster to just five regions of the Myc protein. We examined the consequences of these changes and found, remarkably, that mutations in four out of the five "hot spots" stabilized Myc and (where examined) activated its oncogenicity. These results demonstrate that stabilization of Myc is a common—but not universal—consequence of tumor-derived mutations and strongly implies that these mutations select for a process that facilitates tumor formation.

Finally, in an atypical set of experiments for us, we collaborated with the Muthuswamy laboratory at CSHL to examine how these mutants impact the ability of Myc to function in a three-dimensional (3D) tissue culture model of breast cancer. These experiments were spurred on by our finding that some tumor-associated mutations in Myc alter its ability to signal apoptosis and by reports from other laboratories that epithelial cell polarity temper Myc's pro-apoptotic capabilities. During the course of these studies, we found that Myc and the Myc mutants behave very similarly in 2D and 3D tissue culture systems. We also found that the reported ineffectiveness of Myc in 3D cultures is due to activation of a transcriptional program in 3D that results in the near-complete shutdown of Myc and Myc target gene expression. Thus, although we can show that shutdown of Myc expression is important for the development of normal polarized epithelia, polarity itself does not attenuate Myc activity. These results demonstrate that mechanisms to control Myc expression—be they at the level of transcription or of proteolysis—are critically important for protecting cells against oncogenic insult by Myc and that cell polarity per se is not sufficient to keep Myc at bay.

Control of Transcription by the Ub–Proteasome System

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

Our research in this area uses the yeast *Saccharomyces cerevisiae* to probe the basic connections between the transcription and ubiquitin systems. Two areas of research have been particularly productive in the last year. First, we have continued our studies of the prototypical yeast activator Gal4. We have found that Gal4 is unstable when it activates transcription, that it is destroyed by the Dsg1 Ub-ligase, and that ubiquitylation and/or destruction of Gal4 is important for its ability to productively activate transcription. Recently, this notion was challenged when a competing group reported that when Gal4 activates transcription, it stably associates with chromatin and locks in to a solid complex that cannot be competed off DNA. The notion that Gal4–promoter complexes are static is incompatible with any model in which proteolysis of the activator has an essential role in transcriptional regulation. Using a variety of assays, we have found that Gal4 associates with chromatin in a highly dynamic way and that reports to the contrary are influenced by an artifact in experimental design. These data support the idea that protein complexes involved in transcription are dynamic structures that depend on newly synthesized activators for continual activity.

Second, we examined carefully whether the proteolytic activity of the proteasome is required for *GAL* gene activation in yeast. This is an area of considerable controversy. To resolve this issue, we took two parallel approaches. First, we combined chemical and genetic inhibition of the protease activity of the proteasome and found that *GAL* gene activation is severely attenuated when proteasome function is impaired. Second,

we developed highly selective monoclonal antibodies against 19S and 20S subunits of the proteasome and used chromatin immunoprecipitation and found that when *GAL* genes are activated, both 19S and 20S subunits associate quickly with the active chromatin and coat active genes as if they are moving with polymerase. We also found that both 19S and 20S subunits dissociate from chromatin within seconds of shutting down GAL transcription. Together, these studies strongly suggest that both the proteolytic and nonproteolytic activities of the proteasome are important for gene activation in yeast. We suggest that recruitment of proteasomes to chromatin brings in a myriad of biochemical properties to facilitate multiple steps in the transcription process.

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CANCER: GENETICS

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

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, Princeton, Atwal has modeled the process by which genetic variants, or alleles, evolve. This has recently led to surprising insights about the role in reproduction played by p53, a master tumor suppressor gene. This work also bears on the question of demonstrating recent selective pressures acting on our genomes.

Alexander Krasnitz and colleagues use mathematical and statistical tools to discover key genetic elements involved in cancer and to understand how cancer cells evolve. Array-based comparative genome hybridization, a technique developed in the Wigler lab, and, more recently, sequencing experiments, have revealed subtle patterns of frequent and widespread aberration in cancer genomes. Krasnitz hypothesizes that aberrant genomic loci observed to recur in a range of cancer types are under selection and therefore are enriched in important cancer genes. He has developed a novel, comprehensive methodology to study such "epicenters" and has used it to analyze multiple-genome data sets in breast, lung, colon and liver cancer. The results have been shared with cancer biology labs across CSHL and have been a key enabling agent of functional studies using mouse models and RNA interference. Krasnitz has begun to apply advanced statistical methods to the latest generation of experimental data, which have characterized tumor samples down to the level of single cells. With such data he and colleagues seek to learn how specific cancer types evolve and how cancer cells migrate in metastasis.

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, his team succeeded in developing mouse models of leukemia that accurately predict human responses to drug resistance. In addition, Lowe has adapted RNA interference (RNAi) technology to produce animal models in which genes can be switched on and off in a spatial, temporal, and reversible manner, and he has used this to identify and characterize new genes that modulate tumor cell responses to chemotherapy. Lowe also has spearheaded a collaborative effort involving four other CSHL labs to identify novel tumor suppressor genes. He combined his earlier integrated oncogenomics approach on human liver cancers with RNAi-based screening in a mouse model. This brought to light nearly two dozen previously unknown tumor suppressor genes. Lowe and colleagues also continue to study cellular senescence, a potentially powerful mechanism for suppressing tumor formation. They have demonstrated that senescence helps limit wound-healing responses in liver disease, a finding that suggests senescence has functional relevance, in addition to cancer, in the maintenance of tissue homeostasis following damage.

Robert Lucito has had an important role along with Michael Wigler in developing innovative technologies, including RDA, ROMA, MOMA and CGH, that have proven valuable to cancer researchers worldwide. As an experimentalist, Lucito conducts studies using these techniques to detect copy-number changes in large sets of human ovarian and pancreatic cancer tissue samples. His 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 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. 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 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 p63 proteins, which regulate development, tumorigenesis, cellular senescence, and aging, in vivo. This year, in model mice, they succeeded in halting the growth of malignant tumors in which p53, a master tumor suppressor, was missing. The key was increasing production of one of the proteins encoded by the p63 gene, called TAp63. This could point to new ways of treating malignancies with p53 deficiencies, which tend to be very aggressive and refractory to treatment.

Scott Powers' work focuses on gene mutations that cause cancer and factors that influence responses to specific anticancer drugs. His lab uses technologies that probe the entire genome to identify candidate cancer genes and evaluate their functional role in cell transformation and tumor biology. They also use whole-genome technologies to guide development of novel cancer diagnostics and therapeutics. Using DNA copy-number analysis, the Powers group pinpoints novel amplified oncogenes and then applies functional studies to address the validity of candidate genes and the mechanisms by which they are implicated in oncogenesis. They have successfully applied this approach in liver, colon, and lung cancer. Powers has had an important role, along with Scott Lowe and other investigators, in the development of a distinctive CSHL approach to functional study of cancer genes. Called integrative oncogenomics, it is a rapid, large-scale screen for genes that are deleted in human cancers and suspected of being tumor suppressors. An initial demonstration of the approach, which worked with a list of 360 candidate human liver cancer genes identified by Powers, culminated in the discovery, in mice, of 13 previously unknown tumor suppressors whose deletion resulted in aggressive cancers.

Michael Wigler's group uses methods for comparative genome analysis to study cancer and other human genetic diseases. These methods (called ROMA and MOMA) evolved from an earlier technique called RDA, 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 are engaged in clinical studies with major research hospitals to discover mutation patterns predicting treatment response and outcome. In this connection, they are also developing methods for the analysis of the genomes of single cells. In collaboration with Jim Hicks and Gregory Hannon, 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 a Center for Quantitative Biology at CSHL, with initial funding from the Simons and Starr Foundations.

QUANTITATIVE BIOLOGY

G.S. Atwal Y. Cai, B. Fendler, M. Monaco, F. Schlesinger, A. Sharpe, S. Shringapure, J. Weiss

During the past year, Marcela Monaco, a former postdoc with Lincoln Stein, was hired as a consultant from July to October. Suyash Shringapure, a summer research intern, returned to Carnegie Mellon University to resume his graduate studies at the Computer Science department, but he continues to collaborate with Bud Mishra and myself on a project we initiated at CSHL. Felix Schlesinger, who worked as a graduate rotation student, has accepted a position in the lab of Thomas Gingeras. Ying Cai completed a graduate rotation and has since accepted a position in my lab. Bernard Fendler, having recently been awarded a doctorate in theoretical physics, was hired at the end of the year as a postdoctoral researcher. Joshua Weiss started work as a volunteer as part of the Partners for the Future program. Alexandra Sharpe worked as a volunteer in the lab from July to October.

Cancer Risk and Haplotype Structure of the *MDM4* Gene

A large body of evidence strongly suggests that the p53 tumor suppressor pathway is central in reducing cancer frequency in vertebrates. The protein product of the haploinsufficient mouse double minute 2 (*MDM2*) oncogene binds to and inhibits the p53 protein. Recent studies of human genetic variants in p53 and MDM2 by our lab have shown that single-nucleotide polymorphisms (SNPs) can affect p53 signaling and confer cancer risk, and they suggest that the pathway is under evolutionary selective pressure. We recently analyzed the haplotype structure of *MDM4*, a structural homolog of *MDM2*, in several different human populations. Unusual patterns of linkage disequilibrium (LD) in the haplotype distribution of *MDM4* indicated the presence of candidate SNPs that may also modify the efficacy of the p53 pathway. Association studies in five different patient populations revealed that these SNPs in *MDM4* confer an increased risk for, or early onset of, human breast and ovarian cancers in Ashkenazi Jewish and European cohorts, respectively. This not only implicates *MDM4* as a key regulator of tumorigenesis in the human breast, but also exploits for the first time evolutionary-driven linkage disequilibrium as a means to select SNPs of p53 pathway genes that might be clinically relevant.

Regulation of Human Fertility by SNPs in the p53 Pathway

The tumor suppressor protein p53 has an important role in maternal reproduction in mice through transcriptional regulation of leukemia inhibitory factor (LIF), a cytokine crucial for blastocyst implantation. To determine whether these observations could be extended to humans, a list of SNPs in the p53 pathway that can modify the function of p53 was assembled and used to study their impact on human fertility. The p53 allele encoding proline at codon 72 (P72) was found to be significantly enriched over the allele encoding arginine (R72) among in vitro fertilization (IVF) patients. The P72 allele serves as a risk factor for implantation failure. LIF levels were significantly lower in cells with the P72 allele than in cells with the R72 allele, which may have contributed to the decreased implantation and fertility associated with the P72 allele. Selected alleles in SNPs in *LIF, Mdm2, Mdm4*, and *Hausp* genes, each of which regulates p53 levels in cells, were also enriched in IVF patients. Interestingly, the role of these SNPs on fertility was much reduced or absent in patients older than 35 years of age, indicating that other functions may have a more important role in infertility in older women. The association of SNPs in the p53 pathway with human fertility suggests that p53 regulates the efficiency of human reproduction. These results also provide a plausible explanation for the evolutionary-positive selection of some alleles in the p53 pathway and demonstrate the alleles in the p53 pathway as a good example of antagonistic pleiotropy.

Fine-scale Detection of Recent Selection Signatures Using Haplotype Entropy

Creation of a coherent genomic map of recent selection is one of the greatest challenges for a better understanding of human evolution. Various methods have provided the means for genome-wide linkage disequilibrium analysis; however, there is still a gap for scanning with short-intermediate resolution. Entropy, a measure of disorder in physics, has been proposed as a new concept to measure genetic diversity and strength of linkage disequilibrium of sequences. We produced a fine-scale genomic scan using haplotype entropy. The analysis with a median resolution size of ~23 kb for HapMap SNPs data of African and Caucasian populations depicted new recent selection signatures, as well as known signatures. Gene-enrichment analysis on the signatures showed associations with diverse diseases such as cardiovascular, immunological, neurological, skeletal, and muscular diseases. The novel entropy framework would be of value for deciphering recent evolutionary histories of humans.

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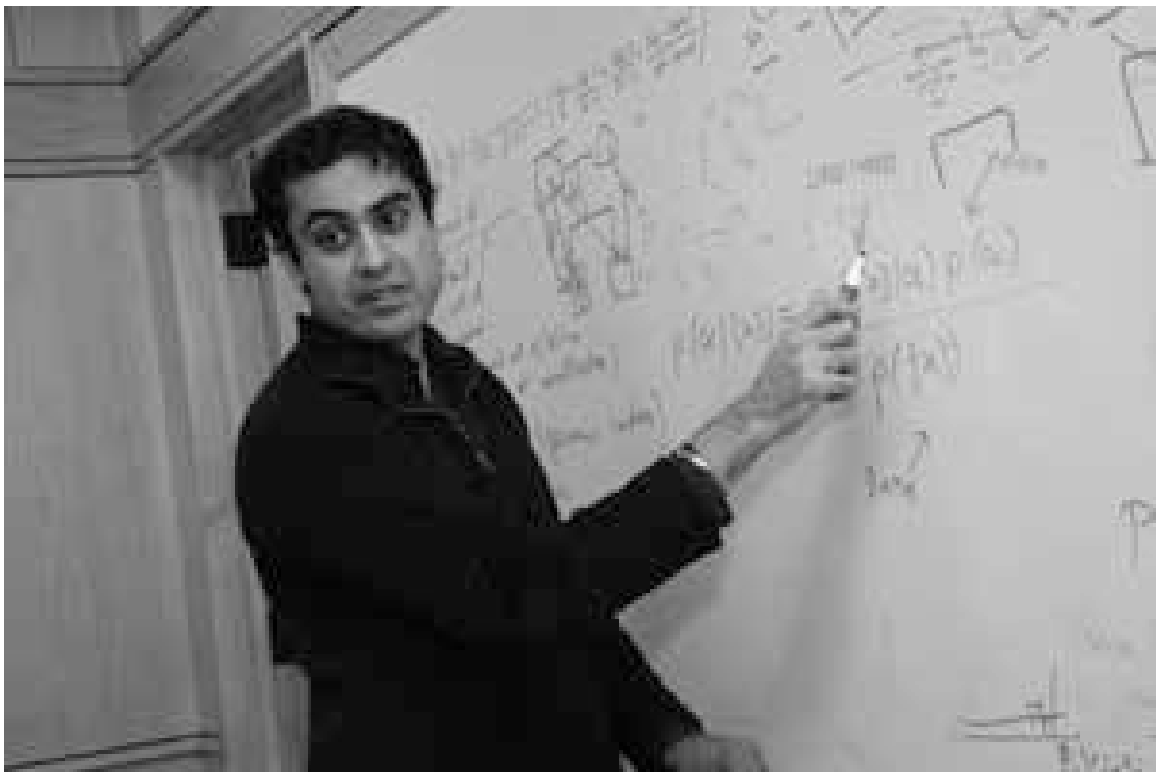

Gurinder "Mickey" Atwal

RNA INTERFERENCE MECHANISMS AND APPLICATIONS

G. Hannon

A. Aravin, J. Brennecke, D. Bressan, R. Burgess, A. Canela, K. Chang, S. Cheloufi, S.W. Chi, J. Colagiola, B. Czech, C. Dos Santos, Y. Erlich, M. Evgenev, K. Fejes Toth, A. Gordon, P. Guzzardo, A.D. Haase, X. He, N. Hiramatsu, E. Hodges, I. Hotta, I. Ibarra, F. Karginov, M. Kudla, C. Malone, K. Marran, A. Molaro, F. Muerdter, O. Navon, J. Preall, C. Rebbeck, F. Rollins, R. Ronen, M. Rooks, A. Rosebrock, N. Rozhkov, E. Rozhkova, N. Shostak, V. Sotirova, O. Tam, P. Theekat, E. Tonin, V. Vagin, A. Valentine, E. Wagenblast, Y. Yu, E. Zelentsova

Our lab focuses on three major areas of biology. For the past decade, we have sought to understand the biological roles of small RNAs and the underlying mechanisms by which they operate. We have identified and characterized many of the major biogenesis and effector complexes for small interfering RNAs (siRNAs) and microRNAs (miRNAs), including Dicer, RISC (RNA-induced silencing complex), and elements of the microprocessor. During the past several years, we have focused on the roles of small RNAs in germ cells, which tend to have the most elaborate set of small RNA pathways of any cell type. This led to the discovery of an essential role for pseudogenes in producing small RNAs that are critical for proper oocyte development and to the discovery of an elegant small-RNA-based immune system that guards the genome against transposable elements. The latter system incorporates another small RNA class, Piwi-interacting RNAs (piRNAs), into an adaptive cycle that both responds to transposon challenge and can communicate epigenetic information about that challenge from parent to progeny. The Hannon lab also strives to understand the biology of cancer cells, with a focus on breast and pancreatic cancer. Here, we are interested in the roles of small RNAs as oncogenes and tumor suppressors and in exploiting the RNA interference (RNAi) libraries that we have developed to identify new therapeutic targets for specific disease subtypes. Finally, we are taking genetic approaches to understand the biology of resistance to currently used targeted therapies. The third component of the laboratory exploits the power of next-generation sequencing to understand the biology of the mammalian genome. Our efforts range from the identification of new classes of small RNAs to understanding human evolution and diversity. Most recently, we have placed a major emphasis on the evolution of the epigenome and its role in driving cell-fate specification. Highlights of scientific progress during the last year are given below.

The past year has seen a number of changes in the members of the Hannon lab. Ingrid Ibarra, Xingyue He, Colin Malone, Oliver Tam, Ikuko Hotta, and Yaniv Erlich successfully defended their theses and were awarded Ph.D. degrees. Yaniv was recognized by the coveted Weintraub Award for his thesis work. Kata Fejes-Toth and Alexei Aravin both departed for faculty positions at the California Institute of Technology. Michelle Rooks departed to begin her Ph.D. studies. Angelique Girard left for a postdoctoral fellowship at Harvard, and Monica Dus for New York University. Vihra Sotirova left following the birth of her first child, and Nobu Hiramatsu departed to return to studies on ER stress. We were joined by several students—Elvin Wagenblast, Antoine Molaro, Felix Muerdter, Ben Czech, and Dario Bressan—and by several postdoctoral fellows—Adam Rosebrock, Sun Wook Chi, Jon Preall, and Yang Yu. Pramod Theekat, Amy Valentine, and John Colagiola also joined the lab. Elena Tonin also came as a visitor from the lab of a former CSHL colleague, Roberta Maestro, and Debbie Goodman and Jacqueline Cortes spent brief stints in the lab as undergraduate researchers.

The Search for Genes Involved in piRNA Biogenesis and Function

A.D. Haase, P. Guzzardo, F. Muerdter, B. Czech

piRNAs together with Piwi-clade Argonaute proteins constitute an evolutionary conserved germline-specifc small RNA silencing system that is implicated in gene silencing, particularly of repetitive elements, germline differentiation, and maintenance of germline stem cells; mutations in Piwi almost universally lead to sterility and germ cell loss.

We are working to identify and characterize genes involved in piRNA biogenesis and function in a *Drosophila* ovary somatic sheet (OSS) cell line. OSS cells, like ovar-

ian somatic stem cells and follicle cells, express Piwi and its associated piRNAs. To screen for genes involved in piRNA biogenesis and effector steps, we are establishing direct and indirect sensor systems. These sensor systems are based on direct detection of piRNAs and transcripts of transposable elements by real-time polymerase chain reaction (PCR), and sensor transgenes, respectively. We aim to perform a genome-wide RNAi screen. In addition to this genome-wide study, we are pursuing two independent approaches to determine sets of genes for a smaller, more targeted screen. To this end, we have compared the transcriptome of OSS cells to that of S2 cells, which do not express Piwi proteins or piRNAs, and are analyzing Piwi protein complexes by mass spectrometry.

Overall, our study aims to advance our understanding of how genome integrity is guarded by small RNAs in the germline.

Arginine Methylation as a Molecular Signature of the Piwi Small RNA Pathway

V. Vagin, A. Aravin

In animals, members of the Argonaute protein family are subdivided into two clades, Ago and Piwi, based on their sequence and expression pattern. We comprehensively characterized Piwi-associated proteins in mouse germline cells and found that Piwi associates with the PRMT5/WDR77 complex, which is known to methylate arginine residues in numerous proteins. Piwi complexes also contain several proteins with Tudor domains; these domains bind to symmetrically methylated arginine. Piwi proteins harbor several methylated arginines, and these residues are critical for interaction with Tudor proteins. Methylated arginines are clustered at the amino termini of Piwi proteins and are absent in Ago members, providing a molecular mechanism that differentiates between members of the two clades. Indeed, Piwi, but not Ago, members faithfully interact with Tudor proteins in a heterologous cell culture system, and several Tudor domain proteins localize to nuage granules together with Piwi in germ cells. Tudor proteins might therefore provide the platform necessary for assembly of numerous proteins that function as a complex in the Piwi pathway.

Conserved Endonucleolytic Processing Events in Mammalian mRNAs

F. Karginov

Argonaute proteins are guided by their miRNA partners to mRNA targets, leading to regulation of the mRNA in a spacially and temporally controlled manner. The majority of miRNA–mRNA interactions do not lead to Argonaute-directed cleavage but instead lead to to destablilzation of the mRNA in a way that does not depend on Ago catalysis. Nevertheless, the catalytic potential of Ago2 is deeply conserved. In an effort to understand the pressure for this conservation, we searched for miRNA-directed Ago cleavage targets by global RACE (rapid amplification of cDNA ends) of mRNAs in wild-type and Ago2 mutant embryonic stem (ES) cells. We identified a number of previously unknown miRNA cleavage targets, but we also saw a wide array of mRNA processing sites that could not be explained by Ago. Another RNAi pathway component, Drosha, had previously been implicated in the regulation of one gene, *DGCR8*, by cleaving a site that resembles a miRNA precursor. We found a number of other Drosha cleavage sites within mRNAs, a subset of which can be demonstrated to regulate mRNA levels. Even after we accounted for all Drosha and Ago2 cleavage sites, a large number of processing sites remained, and many of these were evolutionarily conserved in othologous mouse mRNAs. Considered as a whole, our findings not only reveal a previously unappreciated breadth in the roles of RNAi family enzymes in mRNA cleavage, but also suggest that mRNAs are subject to a surprising diversity of endonucleolytic, posttranscriptional processing events. The path toward understanding the precise biological impact of these processing events will likely require our linking individual cleavage sites with the nucleolytic complexes that generate them.

The Biogenesis of "Amir," a Blood-specific miRNA, Requires Argonaute 2 Cleavage Activity Independently of Dicer

S. Cheloufi

In mammals, Argonaute 2 is the only endonuclease that loads small RNAs (miRNAs or siRNAs) and uses them as complementary guides to slice target mRNAs. The couple of miRNAs that have been demonstrated to cleave perfect complementary targets, together with the discovery of endogenous siRNAs in the female germline, hints to the important role of the slicer activity during development. However, the direct biological role of Argonaute catalysis has not been studied in the whole organism. Here, we show that mice harboring a catalytically inactive Argonaute survive to term but manifest anemia and die shortly after birth. This is the first demonstration of the essential role of Argonaute catalysis somatically during development. The maturation of the small RNA–

Argonaute gene-silencing complex is orchestrated through a series of enzymatic steps acting on precursor RNA molecules. miRNAs depend on Drosha and Dicer for maturation, and siRNAs depend on dicer only. Here, We demonstrate that mammalian Argonaute 2 is loaded with the precursor of a well-conserved blood-specific miRNA following Drosha processing of its primary transcript, bypassing the dicer step. We further show that its slicer activity is required for the maturation of this miRNA. This is the first demonstration of the requirement of Argonaute catalysis in miRNA biogenesis, a notion that can be exploited to generate potent silencing complexes. The function of this small RNA has been reported to be essential for erythropoiesis in the mouse and zebrafish, similar to the mouse model described here. Further genetic and biochemical studies will help unravel other specific developmental processes that lead to the conservation of the catalytic function of the Argonaute protein in mammals.

Hierarchical Rules for Argonaute Loading in *Drosophila*

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

Animal miRNAs are derived from structured RNA precursors, which are processed through the sequential action of two RNase III family enzymes, Drosha and Dicer, to yield double-stranded RNA duplexes of 21–23 nucleotides in length. The vast majority of miRNA duplexes are intrinsically asymmetric in character, with one strand, called the miR strand, differentially entering RISC to recognize and regulate the expression of target mRNAs. The other strand, called miR*, has generally been viewed as a mere by-product of miRNA biogenesis. In *Drosophila*, miRNAs preferentially occupy one of its two Argonaute family members, Argonaute-1 (AGO1), whereas siRNAs, either derived from endogenous double-stranded RNAs or exogenous RNAi triggers, enter Argonaute-2 (AGO2).

By deeply sequencing AGO1- and AGO2-associated small RNAs, we observed that miR* strands are among the most abundant individual species in AGO2 RISC. This is supported by many miR*s bearing 2′-*O*-methyl modifications that mark AGO2-associated species. Through this association, miR* strands can repress target mRNAs carrying perfectly complementary sites both in cultured cells and in animals. Although both miRs and miR*s are Dcr-1 products, loading of miR*s into AGO2 depends additionally on canonical siRNA loading factors, Dcr-2 and R2D2.

These results suggest that a single miRNA precursor can produce two potentially functional small RNAs, with each strand having distinct intrinsic loading signals. By examining the loading patterns of manipulated miRNA and siRNA duplexes, we infer a set of hierarchical rules for AGO loading that incorporate both previously proposed structural determinants and thermodynamic asymmetry.

miRNAs in Normal and Cancer Stem Cells

I. Ibarra [in collaboration with the Wicha lab at the University of Michigan]

The elucidation of the stem and progenitor cell compartments in the mammary epithelium, and uncovering their potential role in tumorigenic processes, has been the focus of my research. The mammary gland is a dynamic tissue that undergoes various morphological transitions during puberty, pregnancy, lactation, and involution in response to ovarian-secreted hormones. Although there is evidence for the existence of stem cells within the mammary epithelium, the location of these cells during mammary gland development remains elusive. The expression of a highly conserved miRNA, *let-7*, in differentiated cell types provides a way to isolate less-committed cell types. We have generated a *let-7* sensor mouse model that allows in vivo tracking of primitive compartments in the mammary gland, intestinal crypts, and bone marrow. miR sensor technology has allowed us to access the temporal and cell-type-specific expression pattern of a single miRNA that has a key role in stem cell biology.

In parallel, we have worked with the Wicha lab at the University of Michigan to test the relevance of miRNA in human cancer stem cells. We found that transiently expressing either of two miRNAs, *let-7* or *miR-93*, whose levels are strongly reduced in stem cells, negates the ability of human breast cancer cell lines to form tumors in mice. This provides strong evidence in favor of the cancer stem cell hypothesis and suggests potential strategies for addressing this compartment in human cancer.

miRNAs as Therapeutic Targets in Breast Cancer

C. Dos Santos

Since November 2008, we have been testing the delivery of a new type of molecule that blocks miRNA function to murine breast tumors. Mice bearing breast tumors

were treated with locked nucleic acid (LNA) antagonists or control LNAs via intravenous injection. Live-image analysis of tumors, which express a fluorescent protein under the control of target miRNA, were quantified after treatment. Preliminary data suggest that there was efficient LNA delivery to breast tumors, and experiments to further test effects on tumor prevention are being performed.

Moreover, we are interested in identifying novel mechanisms regulating mammary gland stem cell biology. First, we are using unbiased gene knockdown screens to identify essential factors that control stem cell self-renewal. Second, we are searching for modifications occurring at the DNA level in different stages of mammary development—menarche, early age pregnancy, and late age pregnancy—and their correlation with breast cancer predisposition.

Tumor-associated Macrophage in Breast Cancer

C. Rebbeck

Macrophage cells are a key component of the body's immune system. They are frequently found in tumor tissue, and in breast tumors, for example, these cells can comprise up to 80% of the tumor mass. These tumor-associated macrophages (TAMs) are considered to be in an altered state of activation and are thought to actually aid tumor growth, development, and metastasis. Our research has involved identifying and isolating these TAMs from breast tumors in order to create a miRNA profile to look for differences between these and other activated macrophages in the hope of better understanding what makes these TAMs behave in a tumor-promoting manner.

In addition, we have been designing a construct to allow labeling of individual cells with various color combinations to use in the study of tumor seeding, metastasis, and development.

Pathways Affecting the Effectiveness of the EGFR Blockade in Lung Cancer Therapy

F. Rollins [in collaboration with R. Sordella, Cold Spring Harbor Laboratory]

During the past year, we have been focusing on elucidating the mechanisms underlying resistance to specific, FDA-approved, chemotherapeutics. Using a cell culture model of non-small-cell lung cancer, we have screened a sparse representation of the genome, including a selected set of genes believed to be involved in cancer. Currently, we are in the validation stages, having identified multiple targets that could significantly contribute to drug sensitivity and perhaps be used as combination therapy to treat even resistant patients.

Structural Variation in Human Cancer

A. Canela

During the past year, we have been developing a methodology to characterize structural variations in cancer cells at a single-base resolution level by next-generation sequencing. First, we focused on regions containing chromosomal rearrangements identified by comparative genomic hybridization (CGH) analysis (in collaboration with the Wigler lab at CSHL) in breast cancer, to evaluate the use of copy-number variations as landmarks of complex chromosomal rearrangements. These regions are purified from tumor genomes by array capture, and the nature of chromosomal rearrangement is identified by sequencing. Second, we used large-insert genomic libraries as an unbiased strategy. Next-generation paired-end sequencing maps both ends of each fragment of the library, allowing the study of chromosomal rearrangements and copy-number variation (structural variation) in the tumor genome. This methodology will allow us to build a structural variation map of tumor genomes and study the repercussion of chromosomal rearrangements in the tumorigenesis.

Finding the Causative Mutation of Joubert Syndrome Type 2 in Ashkenazi Jews by Whole-exome Sequencing

Y. Erlich, E. Hodges

Joubert syndrome type 2 is a devastating disease that is characterized by cerebellum malformation, ataxia, ocular problems, and kidney insufficiency. We studied several Ashkenazi families that suffer from Joubert syndrome in collaboration with Dor-Yeshorim, a Brooklyn-based foundation that focuses on prevention of genetic diseases in the ultra-orthodox Jewish community. Although there are nine genes that are linked to Joubert in other populations, none of these genes were mutated in our samples. We used whole-exome sequencing of a mother and affected daughter to identify possible variations that may cause the disease. On the basis of the profile of the incidents, our working assumption was that the disease is recessive and that there is a single founder mutation. According to this assump-

tion, we narrowed 20,000 mutual variations in the mother and daughter to a list of 39 potential variations. We further narrowed down the region by autozygosity mapping to a 3-Mb area on chromosome 11. We found only a single possible variation in a gene called *TMEM216* that encodes a small transmembrane protein with 88 amino acids. We found that a single-nucleotide polymorphism (SNP) causes arginine to be replaced by leucine in position 12 in both copies of the affected daughter. In collaboration with the Hadsa Medical Center (Israel), we performed tests on other Ashkenazi patients, which indicated that this is the causative mutation of the syndrome. This is one of the first studies that uses whole-exome sequencing to identify causative mutation of Mendelian disorders. The Dor-Yeshorim Foundation routinely tests this mutation in their carrier screening program.

DNA Sudoku

Y. Erlich, K. Chang, A. Gordon, R. Ronen, O. Navon, M. Rooks

Although next-generation sequencing technologies have greatly increased sequencing capacity, multiplexing libraries composed of a large number of specimens is still challenging. Current multiplexing schemes encode the specimen identity by appending unique DNA bar codes to each one. These approaches scale poorly because bar-code synthesis and library preparations for a large number of specimens are cumbersome and expensive.

We devised a different approach, which is based on combinatorial pooling: Tens of thousands of specimens are first grouped into mere hundreds of pools according to a designated mathematical pattern. Then, the pools, instead of the individual specimens, are bar-coded. This strategy, which is deeply rooted in information theory (and solving Sudoku puzzles), is applied to "needle in a haystack" sequencing scenarios, such as carrier screens and finding rare mutations.

In the past year, we used this strategy to genotype 500,000 bacterial colonies, and we showed that with only a single Illumina GAII run, and 384 bar codes, one could genotype a batch composed of 40,000 bacterial colonies. Recently, we began a collaboration with the Dor-Yeshorim Foundation, one of the largest carrier screen programs in North America, to test the method for high-throughput carrier screen for rare Mendelian diseases. On the basis of extensive simulations, we envision an ultrafast, inexpensive, and scalable carrier screen strategy using our approach.

Mechanisms Driving Human Variation and Evolution

R. Burgess

Eukaryotic germline cells undergo meiotic recombination, in which homologous pairs of parental chromosomes are broken and rejoined to form the single-composite chromosomes that are passed through egg or sperm to progeny. By this means, alleles at physically linked loci on the same chromosome may be "reshuffled" in a population, allowing combinations of novel genes that arose in different ancestors to be brought together in one individual. This critical evolutionary process remains poorly understood—in particular the molecular specification of *recombination hot spots*, places in the genomic DNA where the breaks preferentially occur. Working initially with mouse models, we have been developing assays to measure the distribution of recombination events across the mammalian genome, using sperm cells to obtain a large sample of meiotic outcomes in a single individual. These assays will provide the first experimental platform to analyze the specification of meiotic recombination events in living mammals.

Comparative Analysis of the Modern Human and Neandertal Proteomes

E. Hodges [in collaboration with the Pääbo lab at the Max-Planck Institute]

During the past several years, we have developed and refined methods for purifying specific genomic regions by in situ array capture. Despite the increasing power of next-generation sequencing technologies, determining the sequences of extinct organisms from ancient DNA samples remains a daunting task, in part because these samples are invariably contaminated with large amounts of bacterial and fungal DNA. Working with Svante Pääbo at the Max-Planck Institute in Leipzig, we demonstrated that we could purify hominid DNA from libraries made from ~40,000-year-old Neandertal bones, with an enrichment of up to 50,000-fold for areas of interest. Using this procedure, we essentially reconstructed the protein sequences of Neandertal and compared them with those of modern humans. This process focused on sites at which chimp and human differed (~5 million years of divergence) and where modern humans had a different coding variant from the ancestral chimp allele. Using this approach, we have sequenced ~14,000 protein-coding positions that have been inferred to have changed on the human lineage

since the last common ancestor shared with chimpanzees. We identified 88 amino-acid substitutions that have become fixed in all humans since the divergence from the Neandertals.

Genome-wide Analysis of DNA Methylation during Stem Cell Differentiation and Development

E. Hodges, A. Molaro, O. Tam [in collaboration with the Smith lab at the University of Southern California, and the Raffii lab at Cornell]

The publishing of several mammalian genomes in recent years has advanced our understanding of the underlying genetic programs that govern growth and development. However, it is increasingly clear that additional layers of information, broadly known as epigenetics, are crucial to the establishment and maintenance of these genetic programs throughout the life of the organism. An example of this is DNA methylation, where cytosine nucleotides of a gene promoter are methylated enzymatically. This nucleotide modification has been associated with the formation of heterochromatin and the subsequent repression of the surrounding gene. Although there have been hints of dramatic changes to DNA methylation during growth and development, genome-wide analyses of these epigenetic marks have begun to appear only in recent times. We present three projects here that aim to characterize the epigenetic landscape (DNA methylation state) of mammalian cells during the differentiation of the hematopoietic lineage, maturation of male germ cells, and in the early developing mouse embryo.

Self-renewal is essential for the homeostasis and lifetime maintenance of many organ systems. This process is dependent on rare populations of adult stem cells, whose key features include multilineage potential and repopulating capacity. Blood cell development is driven by the successive restriction of cell fate as multipotent hematopoietic stem cells (HSCs) give rise to all mature red and white blood cell types. We have generated genome-wide, single-base resolution maps of DNA methylation in purified cell populations derived from human and chimpanzee peripheral blood. Specifically, using whole-genome shotgun bisulfite sequencing, we are comparing methylation states in long-term HSCs (the self-renewing cells), mature B lymphocytes, and neutrophils, representing terminally differentiated lymphoid and myeloid cells, respectively. These data will provide insight into differential methylation patterns that influence lineage restrictions while also revealing epigenetic relationships between two closely related primates.

During mammalian development, DNA methylation is erased and reestablished in germ cell development. To achieve this, we successfully generated and sequenced bisulfite-treated genome-wide libraries of murine male germ cells. These cells show 60%–70% methylated CpGs, indicating that de novo methylation acts almost upon the entire genome. When the analysis is extended earlier in development, we found that DNA methylation is drastically reduced to a level of 4% in primordial germ cells. We are also analyzing DNA methylation in the germ cells of humans and chimpanzees to uncover conserved CpG islands and investigate their evolutionary dynamics.

One of our current focal points is to uncover the patterns of remethylation, especially in repetitive sequences, that are epigenetically silenced in the germline. We are also further investigating the role of the piRNA pathway in the targeting of de novo methylation of various transposons by comparing and contrasting the methylation profiles of germ cells between MILI mutant (piRNA-deficient) and wild-type animals.

Early (preimplantation) embryo development is another stage where DNA methylation states are dramatically altered in the mouse. Previous low-resolution studies revealed a global decrease in DNA methylation following fertilization until the eight-cell embryonic stage. This is then followed by a global remethylation of cytosine to levels comparable to those of adult organisms. Although informative, these earlier studies fail to combine a nucleotide-level resolution with a genome-wide approach. To address this shortcoming, we will perform deep sequencing (using the Illumina Genome Analyzer platform) on bisulfite-treated DNA samples from preimplantation embryos to assess their genome-wide DNA methylation status. In collaboration with the Jackson Laboratory in Bar Harbor, Maine, we have collected more than 1000 embryos from the blastocyst and morula stages of embryonic development, which will be converted into Illumina-compatible bisulfite-treated libraries for deep sequencing. The results will be processed through the bioinformatics pipeline developed by Andrew Smith, which will allow us to identify and characterize the DNA methylation during these critical stages of development. This project would provide valuable insights into the role of DNA mehtylation during early embryonic development, and hints at their possible roles in specifying and regulating pluripotency and differentiation.

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INTEGRATED APPROACHES TO CANCER GENETICS AND BIOLOGY

S. Lowe C. Bialucha, J. Bolden, J. Cappellani, A. Chicas, Y. Chien, L. Dow, S. Ebbesen, C. Fellmann, D. Grace, S. Guo, S. He, T. Kitzing, V. Krizhanovsky, L. Lintault, A. Lujambio, B. Ma, K. McJunkin, O. Mert, C. Miething, Y. Park, P. Premsrirut, A. Rappaport, M. Saborowski, C. Scuoppo, J. Simon, M. Spector, V. Sridhar, N. Tasdemir, M. Taylor, S.J. White, A. Yu, Z. Zhao, 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, Y. Chien, C. Miething, C. Scuoppo, J. Simon
[in collaboration G. Hannon, Cold Spring Harbor Laboratory, J. Pelletier, McGill University, and former laboratory member 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 in understanding how they function in a "tumor-suppressor network."

Much of our current work on apoptosis and survival signaling exploits the E *-myc* transgenic mouse model. This model of B-cell lymphoma, where disruption of the ARF-p53 pathway, or overexpression of the prosurvival gene *Bcl-2*, cooperates with Myc to dramatically accelerate lymphomagenesis. Similarly, prosurvival signaling through the phosphoinositol-3 kinase (PI3K)/Akt pathway can also promote lymphomagenesis. Using a variety of approaches, we have produced a large number of lym-

phoma genotypes in which cell survival is disrupted in various ways and used this to identify and characterize how cancer genotype influences tumor cell responses to cytotoxic and targeted anticancer drugs. Recently, we have collaborated with Ricky Johnstone (Peter McCallum Cancer Center) and Jerry Pelletier (McGill University) to study the impact of histone deacetylase and translation elogation inhibitors on treatment outcome (Ellis et al. 2009; Robert et al. 2009). With Dr. Pelletier, we continue to use the E *-myc* system to study the impact of translational regulators on oncogenesis and translational inhibitors on tumor regression.

We previously used the E *-myc* model to demonstrate that *p53* gene suppression by RNAi, like p53 mutations, could promote Myc-induced lymphomagenesis, providing the first evidence that RNAi could be used to mimic the effects of tumor-suppressor gene loss. These studies involved isolation of hematopoietic stem and progenitor cells from E *-myc* mice, introducing short hairpin RNAs (shRNAs) that suppressed p53 protein and monitoring tumorigenesis following retransplantation into the hematopoietic compartment of recipient mice. This year, by introducing pools of shRNAs into the same system, we performed a positive-selection-based RNAi screen to identify a series of new tumor suppressors that accelerated E *-myc* tumorigenesis (see below). One gene we identified as a potent tumor suppressor was *RAD17*, which acts to mediate DNA-damage responses to replicative stress (Bric et al. 2009). We showed that shRNAs suppressing *RAD17* can attenuate Myc-induced apoptosis and enable Myc-expressing cells to proliferate more rapidly. Remarkably, only partial *RAD17* suppression produced a proliferation and survival advantage, whereas complete *RAD17* suppression was lethal to the cell. Thus, *RAD17* is a haploinsufficient tumor suppressor that appears to mediate hyperproliferative signaling to Myc. Additional screens are currently in progress to identify other tumor suppressors in lymphoma and to identify candidate drug targets whose inhibition may reverse the effects of prosurvival mutations during lymphomagenesis.

Roles and Regulation of Cellular Senescence

J. Bolden, A. Chicas, Y. Chien, V. Krizhanovsky, A. Lujambio, B. Ma, O. Mert, J. Simon [in collaboration with A. Mills, X. Wang, C. Zhang, and M. Zhang, Cold Spring Harbor Laboratory, and M. Narita, Cancer Research United Kingdom, Cambridge]

Cellular senescence was originally described as the process that accompanies replicative exhaustion in cultured human fibroblasts and is characterized by a series of poorly understood markers. Senescent cells remain metabolically active, but they are unable to proliferate and display changes in gene expression that could alter tissue physiology. Although "replicative" senescence is triggered by telomere attrition and can be prevented by telomerase, an identical endpoint can be produced acutely in response to activated oncogenes, DNA damage, oxidative stress, and suboptimal cell culture conditions. These observations have led us to propose that senescence acts in parallel to apoptosis as a cellular response to stress and acts in a similar way to suppress tumorigenesis and mediate responses to chemotherapy.

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 continue 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. This year, we collaborated with the Mills lab to study the role of the p53 family member, p63, in senescence (Guo et al. 2009). We also performed large gene expression and chromatin-binding experiments to identify Rb-regulated genes that are unique to the senescent state (Chicas et al., in press). We showed that Rb preferentially associates with E2F target genes involved in DNA replication and is uniquely required to repress these genes during senescence but not other growth states. Consequently, Rb loss leads to inappropriate DNA synthesis following a senescence trigger and, together with disruption of a p53-mediated cell cycle checkpoint, enables extensive proliferation and rampant genomic instability. Our results identify a nonredundant Rb effector function that may contribute to tumor suppression and reveal how loss of Rb and p53 cooperate to bypass senescence.

We also continue to be interested in the biology of senescence and the impact and fate of senescent cells in tissues. Our interest stems from the observations that (1) cells triggered to senesce undergo dramatic changes in gene expression that lead to a "senescence-associated secretory phenotype" involving the up-regulation of inflammatory cytokines and immune modulators, (2) components of the innate immune system are recruited to senescent cells in tissues leading to their clearance, and

(3) prevention of this inflammatory response leads to the retention of senescent cells in tissues and impacts tumor regression or wound-healing responses. These observations suggest that senescence can act as both a tumor suppressive mechanism and one that can promote certain wound-healing responses, and it does so through both cell-autonomous and non-cell-autonomous mechanisms (Krizhanovsky et al. 2009). We continue to explore the interplay between the immune system and senescence in order to provide insights into this previously uncharacterized form of immune surveillance. Such studies provide our first foray into investigating factors that influence the tumor microenvironment.

Mouse Models of Human Cancer

C. Bialucha, J. Cappellani, S. Ebbesen, D. Grace, A. Rappaport, M. Saborowski, C. Scuoppo, J. Simon, M. Taylor, S.J. White, Z. Zhao, J. Zuber [in collaboration with S. Kogan, University of California, San Francisco, and C. Cordon-Cardo, Columbia University]

In cancer patients, tumors arise in the context of normal and diseased tissue, and it has become overwhelmingly apparent that not all processes relevant to cancer progression and therapy response can be understood from the behavior of cells in a Petri dish. For this reason, our laboratory has continually moved toward studying factors that impact cancer biology and therapy using genetically engineered mouse models, where tumor behavior can be studied in the context of its natural microenvironment. These studies have revealed many new insights into the nature and complexity of the genes and processes that contribute to tumor initiation and progression.

One substantial obstacle limiting the use of mouse models is that their production is time-consuming and expensive. To address this issue, we have incorporated a "mosaic" approach to modeling cancer in mice, whereby genes or shRNAs are introduced ex vivo into stem and progenitor cells derived from different tissues, and the genetically modified populations are retransplanted into recipient mice. Using this approach, one can readily vary the target cell population and the nature of the introduced transgene, enabling the production of tumors with a broad range of genotypes. Moreover, because the cells are transplanted into normal recipients, the animals are mosaics where the developing tumor arises in a normal tissue microenvironment. Such models are extremely flexible, and dramatically reduce the time and cost of producing similar models by intercrossing germline transgenic or knockout strains.

Our first mosaic model, described above, was based on the E -*myc* mouse model of lymphoma, which remains a mainstay of research in our laboratory. This year, we established a variety of new leukemia models based on the same fundamental concepts. We observed that mutations commonly found in concert in human patients cooperate in mouse models to promote acute myeloid leukemia (AML). For example, activating mutations in Ras signaling pathways—one of the most common lesions in human AML—are associated and functionally cooperate with AML1/ETO and MLL fusion proteins as well as loss of p53. We showed that these mosaic models mimic the pathophysiological and molecular features of human malignancies to the best level currently available. We are using these models to provide tractable experimental systems to identify cancer-related genes and novel drug targets and to evaluate new compounds and combinatorial therapies preclinically. Other mosaic mouse models in use or under development include those involving epithelial cancers such as liver, breast, lung, and pancreas. Our goal is to use these for cancer gene discovery and validation efforts (see below).

Integrated Approaches to Cancer Gene Discovery

C. Bialucha, S. Ebbesen, S. He, T. Kitzing, B. Ma, C. Miething, A. Rappaport, M. Saborowski, C. Scuoppo, J. Simon, M. Spector, M. Taylor, S.Z. Zhao, J. Zuber [in collaboration with A. Kraznitz, M. Wigler, J. Hicks, G. Hannon, and S. Powers, Cold Spring Harbor Laboratory]

Based on the premise that a complete understanding of the genetic alterations that occur in human cancers will enable more effective use of existing drugs and facilitate the development of improved therapies, genomic technologies are being used on a large scale in efforts to identify all of the recurrent genetic alterations that occur in human tumors. However, because cancers are inherently genomically unstable, it is often difficult to distinguish between "driver" mutations that contribute to the malignancy and those that are "passengers" with no phenotypic effect from this information alone. To address this issue and speed up the pace at which new cancer genes are identified, we are using our mosaic mouse models to filter through human genomic data and rapidly identify the genes and gene combinations that contribute to tumorigenesis. By combining these models with genomic information from human cancers, we are systematically identifying genes that contribute to the development of several tumor types.

Our efforts at identifying cancer genes were initiated in 2005, when we used mosaic models to functionally characterize oncogenes in a region amplified in human and mouse liver carcinomas. Next, we performed an in vivo RNAi screen to identify new tumor suppressors in liver cancer, leading to the identification of 12 new tumor suppressors. This year, continuing our ongoing collaborations with Drs. Hannon, Kraznitz, Hicks, Wigler, and Powers, we used our E *-myc* mosaic model to screen pools of shRNAs targeting a focused set of cancer-relevant genes for their ability to promote tumorigenesis following transduction into stem and progenitor cells and engraftment into syngeneic recipients. More than 20 candidate tumor-promoting shRNAs were isolated, including those targeting established tumor suppressors (Bric et al. 2009). New tumor suppressors included the DNA-damage-response protein Rad17 (see above), molecules that may have pro-oncogenic activities in other contexts (e.g., Mek1), and others that encode secreted proteins (e.g., Sfrp1).

We also continued to perform follow-up studies on cancer genes that we previously identified, as well as worked with collaborators to implement our mouse systems to characterize new cancer genes. Among these include collaborative studies to explore the function of apoptosis regulators such as cIAP1 (Laska et al. 2009; Ma et al. 2009), the hippo pathway effector YAP (Xu et al. 2009), and mir17-92 in tumorigenesis (Olive et al. 2009). In addition, we worked together with Anne DeJean (Pasteur institute) and John Luk (Hong Kong University) to characterize miR221 and cadherin-17 as potential oncogenes in hepatocellular carinoma (Liu et al. 2009; Pineau et al. 2009). Finally, we worked together with David Largaespada (at the University of Minnesota) to characterize potential liver cancer oncogenes identified by insertional mutagenesis in mice (Keng et al. 2009).

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. Although our studies used mouse models of hepatocellular carcinoma and lymphoma and focused only on focal deletions, we are currently extending this relatively high-throughput approach to other mouse models and are screening shRNA pools targeting genes affected by larger deletions, promoter methylation, and point mutations. Moreover, through exploitation of the emerging libraries of full-length cDNAs, we are performing parallel screens for oncogenes involved in genomic amplifications.

Whereas our cancer gene discovery efforts can use genomic data generated by other investigators, we have developed the in-house capability to identify and characterize copy-number changes in various human cancers and, more recently, are incorporating next-generation sequencing to characterize genes of interest in human cancers. These efforts are producing new hypotheses that can be functionally validated in our mouse models. 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

C. Miething, A. Rappaport, J. Simon, M. Spector, M. Taylor, Z. Zhao, J. Zuber [in collaboration with S. Kogan, University of California, San Francisco, and E. Dolan, University of Chicago]

Our laboratory has a longstanding interest in understanding genetic determinants that influence the cellular response to conventional chemotherapy with the long-term 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.

Last year, we used our new mosaic mouse models of AML (see above) to demonstrate 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 (Zuber et al. 2009). 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. They also indicate that p53 action is central to the response of leukemia to conventional chemotherapy.

Developing rational strategies to target these otherwise refractory leukemias is a major goal of current research. We are attacking this problem through both systematic and global approaches using RNAi to identify genes that are required for the maintenance of these leukemias. We also are using the mouse models as preclinical test systems for new drugs and drug combinations.

Modulation of Gene Expression In Vivo Using RNAi

J. Bolden, J. Cappellani, L. Dow, C. Fellmann, D. Grace, S. Guo, B. Ma, K. McJunkin, C. Miething, Y. Park, P. Premsrirut, M. Saborowski, J. Simon, V. Sridhar, S.J. White, A. Yu, J. Zuber [in collaboration with G. Hannon, Cold Spring Harbor Laboratory, and C. Cordon-Cardo, Columbia University]

Together with Gregory Hannon, we continue to develop improvements in RNAi technology with a specific interest in using this approach to probe various aspects of cancer biology. Using shRNAs based on the mir30 microRNA (miRNA) scaffold, we have developed 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. We also have initiated studies 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 areas of technology development involves the production of transgenic mice. We have previously shown that it is possible to produce transgenic mice based on pronuclear injection of a linear fragment encoding a miR30-based shRNA downstream from the tet-responsive element (TRE). To improve upon this, we produced a second generation of transgenic technology that rapidly generates fully transgenic founders with a defined genomic integration. Using the KH2 embryonic stem (ES) cell system from the laboratory of Rudolph Jaenisch (Whitehead Institute), an improved TRE-shRNA expression cassette was recombined into a docking site at the collagen-A1 locus. 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 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 using these systems to produce animals capable of tissue-specific and reversible suppression of tumor suppressors, drug targets, and essential genes.

In addition to using this system to study shRNAs, we have launched efforts to generate a collection of ES cells expressing tet-regulatable miRNAs using an established Flp/Frt recombinase-mediated cassette exchange system that utilizes our own custom-designed Flp-in targeting vector. All known mouse miRNAs at the time of construction have been inserted downstream from GFP of the "tet-on"–Flp-in targeting vectors, which will eventually allow for regulatable expression of miRNAs in ES cells. Additionally, two different kinds of miRNAs are being targeted: One involves expressing the miRNA in its natural context and the other involves expressing the miRNAs embedded in a mir30 context, which expresses particularly well in this system. This collection of ES cell lines will be made available to the community via the NCI Mouse Models of Human Cancer repository. We anticipate that this resource will serve as a powerful tool for the research community at large.

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K. McJunkin

L. Lintault, L. Dow

REPRESENTATION METHYLATION SEQUENCING ANALYSIS

R. Lucito J. Byrnes S. Kamalakaran
E. Cutter E. Lum
M. Joinnides

In addition to genetic mutations 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 suppressors in cancer. We have previously adapted ROMA (representational oligonucleotide microarray analysis) to methylation detection oligonucleotide microarray analysis (MOMA). This methodology was useful and allowed us to identify genes that were methylated in the tumor cells, but it only gave us a general idea of where in the CpG island the methylation had occurred. In addition, after performing the analysis, sequencing validation was required to determine which of the array measurements accurately identified methylated fragments. With the development of next-generation sequencing technologies, it is now possible to identify the methylation status for the vast majority of CpG islands. Our approach is to sequence MspI representations of the genome. We have developed several different approaches to generate the libraries for sequencing and have decided to prepare Illumina libraries from bisulfite-treated MspI representations.

We are presently sequencing three tumors and two cell lines. We have determined the coverage and the number of sequences required to obtain appropriate coverage of many of the CpG islands and have developed methods to carry out the analysis on the sequencing data. Mapping the reads to the genome is not trivial because the bisulfite treatment essentially changes the genome sequence to only three bases due to the conversion of most cytosines to thymine. We have used a program developed by Andrew Smith, formerly of CSHL, to map the reads to an in silico bisulfite-treated human genome. After mapping the reads, the sequences that map to CpG islands are separated from other sequences; this represents ~20% of the reads. These reads are contigged using the program Velvet to form larger regions for analysis. The data can now be analyzed to determine gene-specific methylation states. Thus far, we have used the data to identify which genes are methylated in the tumor samples. More importantly, we have expression data for these samples and will use these data to determine which region of the CpG island is methylated for genes that have suppression of transcription. It will also be interesting to determine the effect of methylation of island sequences within the coding region of the gene. We plan on increasing the number of samples for two reasons. We will be analyzing a new set of ovarian tumor samples from our collaborator Dr. Douglas Levine from Memorial Sloan-Kettering Cancer Center. The data from these samples will serve as an independent validation of the genes we have found to be methylated from our MOMA results (see below). These data will also give us a much more accurate view of the regions methylated and the purpose of this methylation with respect to gene expression.

Methylome Analysis of Ovarian Cancer

K. Wrzeszczynski [in collaboration with M. Zheng]

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 I or II 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 III, with 5-year survival for stage III or IV 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 in the past few decades, the survival rate has not improved significantly. Currently, the 5-year survival rate 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. Com-

mon 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 relapse will ultimately develop acquired platinum resistance.

We hypothesize that the tumors 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 affects 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 sample sets have associated expression and copy-number data. After performing MOMA on these samples, the data were associated with clinical data. Statistical analysis was performed to identify the methylation differences that are present. These differences can segregate patients on the basis of the clinical variable platinum-free interval (PFI), which is the time from the end of chemotherapy treatment to the recurrence of tumor progression. For gene candidates that are able to segregate the patients on the basis of PFI, expression data were associated to identify those genes whose expression was suppressed presumably by methylation. Thus far, we have identified ~600 genes that we are now subjecting to methylation validation with the gold standard bisulfite sequencing.

A small set of 30 genes was selected for functional validation experiments based on literature analysis for genes that were found methylated. We have developed a culture-based system to screen for genes that affect platinum resistance when expression is suppressed. We have characterized the platinum sensitivity for 14 ovarian tumor cell lines and two normal immortalized ovarian cell lines. Four of these cell lines—one normal, two tumor cell lines with varied sensitivities, and one resistant cell line—were selected. To mimic the transcription suppression caused by promoter methylation, we have used short hairpin RNA (shRNA) clones for the gene candidates. Up to three clones for each gene were used, and to increase throughput, pools of 30 shRNAs each were prepared. Retroviruses for each pool were prepared and used to infect the four cell lines and selected. After regrowth of the cells, they were challenged with Carboplatin for 3 days at the IC_{50} (half-maximal inhibitory concentration). After selection, the cells were harvested, and genomic DNA was prepared and used as a template for polymerase chain reaction (PCR) to amplify the shRNA insert for sequence identification. From the original 30 genes, we identified four genes that can affect platinum sensitivity when expression is knocked down. For one of these genes (*CHD3*), we have moved on to functional analysis. One interesting phenotype is that the suppression of this gene causes the cells to cycle slower. This would be in line with the theory of how many chemotherapies including Carboplatin target faster-growing cells, which would be tumor cells and a number of specialized normal cells in the individual. Interestingly, we have noticed that some of the genes that we found to be methylated in the sample set were actually oncogenes. We found this at first very difficult to explain. However, in light of our findings, it is possible that this is a mechanism that tumor cells use to escape the blanket chemotherapies that are more toxins than molecules that target specific genes or classes of genes. We are continuing this investigation and are now screening all of the candidate genes that we have identified.

Analysis of the *PAK4* Genes Involved in Pancreatic Cancer

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. We performed ROMA and identified copy-number alterations in 82 tumor samples and used these data to identify new alterations. One amplified region on chromosome 19q12 is interesting because there has been confusion in the literature as to which gene in the region is the oncogene with respect to pancreatic cancer. Although this region is amplified in a number of tumors and cell lines, two primary tumors are the most informative for defining the epicenter or common region of mutation. We have previously shown that the p21-activated kinase-4 (*PAK4*) gene was overexpressed at the RNA level, overproduced at the protein level, and overactivated as a kinase in cells with genomic amplification. This protein was also shown by other investigators 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 both expression analysis and pathway analysis to determine whether any pathways were significantly altered in the cells with *PAK4* compared to the cells with the *PAK4* suppressed. We were surprised to find that of the pathways altered by the knockdown of *PAK4,* the most obvious one involving cellular movement or migration was not greatly affected. This may be due to the fact that *PAK4* is actually more important to other pathways that have yet to be discovered. We did find several interesting pathways that were statistically significant, including intracellular transport, regulation of transcription, blood vessel development, and cell cycle regulation. We already know that when PAK4 is activated, cells undergo transformation, and so the pathways affected by *PAK4* must be more complex, involving others in addition to those involved in cellular movement. We will be using pathway analysis to identify candidate genes that can be studied to identify how *PAK4* is onvolved in transformation. We plan on taking advantage of the fact that a portion of signaling through K-ras goes through *PAK4*. We are presently carrying out expression analysis of cells with shRNAs to K-ras so that we can determine the overlap between cells with activated *PAK4* and those with activated K-ras.

Genomic Alterations of Phosphatases

F. Chaudhary [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 signaling 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 have used genomic copy-number data, expression data, and now epigenetic DNA methylation data to determine which PTPs are altered in ovarian cancer. We have also expanded our extension of analysis of PTPs in cancer into the proteins with which PTPs interact. An interesting gene in this class encodes the MTSS1 protein which interacts with a PTP, the receptor-like PTP RPTPδ, and regulates cytoskeletal organization. It is known to be preferentially methylated in several cancers, including breast cancer, and its expression is markedly decreased in ovarian cancer.

We first generated shRNAs to suppress expression of the *MTSS1* gene in cell culture assays, which would mimic the transcriptional repression caused by promoter methylation. Because this gene was selected based only on the comparison of tumor to normal, we did not have a selection for assay development. We surmised that because *MTSS1* possessed an actin-binding domain, the protein might have a role in cytoskeletal rearrangement, cell movement, and invasion. Therefore, we performed an invasion assay with MCF10A mammary epithelial cells, which are used typically as a normal line. We observed that very few cells migrate without the addition of the attractant epidermal growth factor (EGF). Furthermore, RNA interference (RNAi)-mediated suppression of *MTSS1* enhanced migration in the presence of EGF. There is evidence in the literature that in *ERBB2*-positive breast cancer, there is loss of *MTSS1* transcription. To determine whether *ERBB2* overexpression synergizes with loss of MTSS1, we performed an invasion assay in MCF10AN cells. These cells express a chimeric *ERBB2* gene in which the activity of the cytoplasmic PTK domain of *ERBB2* is acutely regulated by a small-molecule dimerizing agent. We noted that the level of invasion is highest in the cells in which *MTSS1* was suppressed and *ERBB2* activated, demonstrating cooperation between these two gene products. Our current data indicate that these effects are mediated via changes in tyrosine phosphorylation regulated by RPTPδ.

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MAMMALIAN FUNCTIONAL GENOMICS

A. Mills Y. Chang W. Li E. Vernersson-Lindahl
G. Horev S. Paul A. Vestin
D.-W. Hwang Y.-E. Son M. Wittinger

The Mills laboratory is focused on mammalian genetics, specifically on identifying and characterizing genes that contribute to human diseases such as those affecting development, aging, and cancer. 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*

CHD5 maps within *1p36*, a region of the genome that has a three-decade history as being deleted in a wide variety of human cancers. Despite the rich body of evidence indicating that one or more tumor suppressors reside in this region, the causative gene had remained a mystery. We identified CHD5 as a potent tumor suppressor by (1) establishing that deletions encompassing *Chd5* predispose to a variety of spontaneous cancers, (2) determining that specific deficiency of Chd5 enhances tumorigenesis in vivo, and (3) demonstrating that *CHD5* is frequently deleted in human glioma. In addition to these loss-of-function studies, we found that mouse models with gain of the interval encompassing *Chd5* have excessive tumor suppression. The finding that CHD5 is a tumor suppressor was further corroborated by other investigators in subsequent work showing that *CHD5* is deleted in neuroblastoma and T-cell acute lymphoblastomic leukemia or mutated in neuroblastoma and breast cancer. The discovery that CHD5 is a tumor suppressor is also consistent with an extensive body of literature indicating that *CHD5* maps within a genomic region commonly deleted in diverse types of human cancer.

The reciprocal gain and loss models that we generated using chromosome engineering were invaluable for determining the mechanism by which CHD5 functions as a tumor suppressor. We found that CHD5 is a master switch for a tumor suppressive network. Chd5 positively regulates the *Ink4/Arf* locus, thereby facilitating expression of p16 and p19—two important tumor suppressors. Compromised Chd5 activity, either by engineered heterozygous deletion of the interval encompassing *Chd5* or by specific depletion of Chd5 using RNA interference (RNAi), cripples the tumor suppressive network, compromising p16/Rb- and p19/p53-mediated pathways, predisposing to cancer. Intriguingly, Chd5 is a predicted chromatin remodeling protein, consistent with our view that it regulates *Ink4/Arf* by maintaining this locus in a transcriptionally competent chromatin state. Indeed, gain of Chd5 dosage enhances expression of p16, p19, and p53, causing compromised proliferation and senescence in cultured cells as well as excessive apoptosis in vivo.

We are currently (1) assessing the spectrum of tumors that develop in mice with compromised 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. Having identified CHD5 as a novel tumor suppressor, we now strive to more fully understand the mechanism whereby CHD5 protects from cancer so that more effective anticancer therapies can be designed in the future.

Function of the *p53* Homolog *p63*

p63 is essential for development. 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. We previously identified the *p63* gene and generated a number of p63-deficient mouse models, which allowed us to determine that despite their similarities, p63 and p53 perform very different functions in vivo: p63 is essential for development of stratified epithelia, whereas p53 is dispensable during embryogenesis but functions as a powerful tumor suppressor in the adult. To explore whether compromised p63 function leads to cancer, we monitored $p63^{+/-}$ mice for spontaneous cancers over a 2-year period. This study demonstrated that mice with reduced p63 are not prone to cancer, further demonstrating the unique roles of p53-related proteins.

p63 links cellular senescence and aging. During the course of the tumor study described above, we realized 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 off p63 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.

TAp63 induces cellular senescence and shuts down tumor growth. A fascinating feature of the *p63* gene (as well as the *p53* and *p73* genes) is that it encodes multiple proteins that fall into the TAp63 and the ΔNp63 classes. This year, we asked which p63 protein class was responsible for senescence. We found that TAp63 was able to trigger senescence that was dependent on the cell cycle regulator p21. By using chromosome engineering to create mice that are specifically depleted of TAp63 proteins, yet still express ΔNp63 proteins, we discovered that senescence in response to oncogenic insults did not occur when TAp63 was absent. This indicates that TAp63 proteins are essential for cellular senescence. TAp63's ability to induce senescence completely blocked the initiation of tumors. Even more strikingly, when we used an inducible expression system to turn on TAp63 in tumors that were already established, senescence was induced, p21 was expressed, and the tumors stopped growing. Importantly, TAp63-mediated senescence could even occur in tumors that did not have p53. Because p53 is absent in the majority of human cancers, this finding suggests that strategies that turn up TAp63 levels could provide a new way to treat human malignancies.

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Emma Vernersson-Lindahl

CANCER GENES

S. Powers C. Cai, M. Chanrion, M. Costanza, C. Eifert, B. Gerdes, J. Marchica, A. Mofunanya, M. Rajaram, K. Revill, E. Sawey, M. Su, T. Wang, J. Zhang, X. Zhou

Despite the considerable progress in cancer research and treatment during the last decade, fundamental questions still exist regarding clinically important cancers including liver cancer and ovarian cancer. Breast cancer to a first approximation is driven by aberrant estrogen, HER2, and phosphoinositol-3 kinase (PI3K) signaling, but what factor has a similar role in liver cancer or ovarian cancer? What are these tumors dependent on that can be exploited by targeted therapeutics? To help answer these questions, this year we became part of a newly formed National Cancer Institute (NCI)-funded network of Cancer Target Discovery & Development Centers. Below, we describe this new project along with our ongoing work in hepatocellular carcinoma (HCC).

Integrative Genomic Discovery of Driver Genes and Oncogenic Dependencies

E. Sawey, C. Cai, M. Costanza, B. Gerdes, J. Marchica [in collaboration with S. Lowe, A. Krasnitz, L. Stein, S. Guo, P. Premisrut, G. Wu, Cold Spring Harbor Laboratory; D. French (Genentech); and R. Finn and S. Orsulic, University of California, Los Angeles]

Cancer genome projects have revealed a tremendous amount of information about structural alterations in specific cancers, but they have provided surprisingly little knowledge about what drives the formation of these cancers. To identify driver genes from the mountain of genomic data, we have been collaborating with Scott Lowe's lab at CSHL to develop relatively high-throughput functional screening methods that use an appropriate mouse model to test the oncogenicity of candidate drivers. Our lab's focus has been on using cDNAs to discover oncogenic driver genes from human amplicons.

Last year, we reported that we were using this approach to discover several new amplified driver genes in human HCC. This year, we completed our genome-wide functional screen in which we identified oncogenic driver genes from recurrent amplified regions in HCC and a follow-up study to determine whether human HCC cells containing these amplified genes show evidence of oncogene dependence. We identified eight completely novel oncogenes that provide new insights into the cellular processes that can drive malignant transformation. One interesting example is POLR1C, which encodes the highly conserved RPA40 subunit of both RNA polymerases I and III (RNA pol I and pol III). Its function can be characterized as "housekeeping," making it problematic to reconcile with known cancer pathways. However, it has long been known that there is an increase in RNA pol I and III transcription in cancer cells. Curiously, RPA40 was shown recently to be tyrosine phosphorylated, and taken together with its role as an amplified oncogene, this suggests that it is a central player in the mechanism driving increased RNA pol I and III transcription in cancer. These results indicate that increased RNA pol I and III transcription can actively drive cancer progression and that these are not merely passive secondary events.

RNA interference (RNAi) has proven to be an extremely effective tool for determining whether a gene commonly altered in cancer still has an important oncogenic role in the fully mature tumor cell. The answer to this question more or less determines whether it is a good therapeutic target. For three of the amplified oncogenes we identified, we performed in-depth analyses using multiple independent RNAi designs to test the effects of silencing these driver genes. In all three cases, RNAi silencing of the target genes inhibited both tumorigenicity and clonal growth of human HCC cells, and this oncogene dependence was only observed in cells harboring the amplified gene. In collaboration with Dorothy French at Genentech and Richard Finn at UCLA, we extended our findings to a candidate cancer drug by demonstrating that *FGF19* amplification is a predictive biomarker of growth inhibition by a neutralizing monoclonal antibody directed against *FGF19*. These results establish that the amplified oncogenome is a valuable resource for systematic functional identification of therapeutic targets.

This discovery of *FGF19* as a driver gene for a significant subset of HCC validates the utility of our func-

tional genomics approach to driver gene discovery. As a result of NCI funding, we expanded our study to ovarian cancer and have begun functional genomic analysis of genes and pathways altered in human ovarian cancer (identified in collaboration with A. Krasnitz and L. Stein, CSHL) using ovarian epithelial progenitor cells provided by Sandra Orsulic's laboratory at UCLA. Our goal in both of these projects is to understand more about the cellular processes that are critical to liver and ovarian cancer and most importantly to discover new strategies for treatment and diagnosis.

RNAi Screens to Discover Tumor Cell Dependencies and Combination Therapy Strategies

A. Mofunanya , C. Eifert, J. Zhang [in collaboration with K. Chang and G. Hannon, Cold Spring Harbor Laboratory; G. Schwartz, Memorial Sloan-Kettering Cancer Center; and K. Bachman and R. Wooster, GlaxoSmithKline]

This year, we continued to use the powerful RNAi screening methodology developed by Greg Hannon here at CSHL to look for combination therapy strategies for cancer drugs, and we also began screening human HCC cell lines to look for tumor cell dependencies. Last year, we reported on a screen for sensitizers to a Polo-kinase inhibitor, a candidate cancer drug, and the discovery that retinoic acid synergized with this compound to kill cancer cells. Unfortunately, an unexpected side effect of that candidate cancer drug caused its clinical development to halt. This year, we have started to screen cancer cells for sensitizers to what turns out to be a much more promising candidate cancer drug, an inhibitor to PI3K, which is an important driver gene in breast cancer. We have also discovered some promising new sensitizers to the standard-of-care drug for treating inoperable melanoma, temozolomide (TMZ). Next year, we hope to report on a clinical trial based on these findings.

Genome-wide Analysis of DNA Methylation Alterations in HCC

K. Revill, T. Wang

As more and more large-scale cancer genome projects take hold, there is less and less for individual laboratories to discover. However, the cancer epigenome is relatively unexplored and likely to be the source of interesting investigations for years to come. We have started to investigate changes that occur in the DNA methylation patterns of HCC using genome-wide technologies. These changes could be just as important as the DNA copy-number changes that we previously characterized. Although this analysis is just beginning, our preliminary findings show that the scope of alterations in HCC differs markedly from that in colorectal cancer, which was not the case for DNA copy-number alterations. In addition to the anticipated increase in CpG-island methylation, we also found that some genes in liver cancer appear to have their CpG islands demethylated instead (Fig. 1).

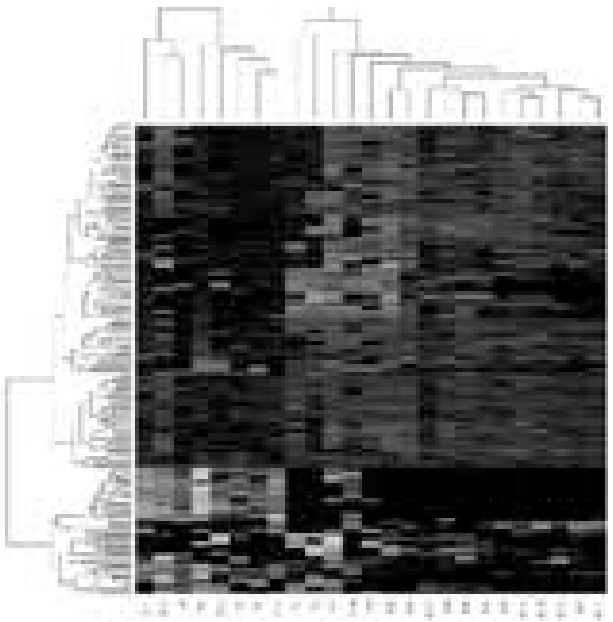

Figure 1. Heat map of the most variant CpG-methylation alterations amongst a set of 12 HCC specimens with matching non-tumorous liver samples. There are two major classes of genes (rows): those that are hypermethylated in a subpopulation of tumors (left-most columns), which form the top group of rows, and those that are hypomethylated in that same set of tumors, which form the bottom rows. Surprisingly, these groups are different than those found altered in colorectal cancer.

How Does the Newly Discovered Oncoprotein FNDC3B Transform Liver Epithelial Cells?

C. Cai

A postdoc in the lab, Chunlin Cai, took on the ambitious goal of determining the mechanism by which a newly discovered oncoprotein, FNDC3B, transforms cells. FNDC3B was discovered in the functional genomic screen for driver genes in HCC described above. For a little more than 2 years, Cai steadfastly attacked this problem using a variety of approaches. Very little was (or still is) known about the normal function of FNDC3B, so he was really starting from scratch. One fact that he managed to establish, with the expert help of Stephen Hearn in the Microscopy Facility, is that FNDC3B localizes to the Golgi apparatus, a rather unusual subcellular localization for oncoproteins. This localization assignment was carefully controlled with independent antibodies to FNDC3B along with the use of green fluorescent protein (GFP) fusions. Mutations that caused mislocalization also destroyed oncogenic function, suggesting that its localization to the Golgi may be essential for its transforming activity. But how? Chunlin tried to obtain a clue to the biochemical mechanisms through identification of interacting proteins by proteomic analysis. This analysis indicated that FNDC3B seems to interact with a bewildering array of proteins, including heat-shock protein 70-kDa protein 1B, lysosomal phospholipase A2, heterogeneous nuclear ribonucleoprotein K, and a protein ignominiously called C20orf70. However, we did not follow up on these proteins because none of them provided compelling clues to the biochemical function of FNDC3B and their interaction seemed potentially nonphysiological. The other approach to find the biochemical mechanism was to start with the oncogenic pathways modulated by FNDC3B. Here, the idea was to survey the cancer pathways for FNDC3B modulation and then to dissect the point at which FNDC3B intervened with the specific pathways. This approach yielded more solid results, which nevertheless were very surprising in that several independent signaling pathways were activated, including Rb1, Akt, PKC, and TGF-β. The latter pathway was examined in detail, and we found that the activation occurred at the level of receptor activation, but not through the production or stimulation of ligand. Although these results were interesting, we did not solve the problem, and the results point out the potential difficulty in determining oncogenic mechanisms. We have learned a lesson from this and will reserve future explorations of the oncogenic mechanism for when it can help move forward a truly promising therapeutic target.

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COPY-NUMBER ANALYSIS AND HUMAN DISEASE

M. Wigler C. Algieri, T. Baslan, M. Bekritsky, S. Chakraborty, K. Cook, Y. Eberling, D. Esposito, V. Grubor, I. Hakker, J. Hicks, J. Huang, R. Kandasamy, J. Katz, J. Kendall, A. Krasnitz, Y.-H. Lee, A. Leotta, D. Levy, S. Marks, J. Meth, N. Navin, M. Oswald, D. Pai, M. Riggs, L. Rodgers, M. Ronemus, J. Troge, Z. Wang, 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 (Lucito et al., *Genome Res 13:* 2291 [2003]; Sebat et al., *Science 305:* 525 [2004]). Such "copy-number" variations, or CNVs, can arise somatically or in the germline. Somatic variations are often seen in cancer and distinguish cancer cells from the normal cells of the body. They provide clues for the origin and behavior of the cancer (Hicks et al., *Genome Res 16:* 1465 [2006]) and possibly its early detection. Analysis of cancer genomes at the single-cell level will enable us to better study how cancers evolve (Navin et al., *Genome Res 20:* 68 [2010]) and provide new tools for clinical evaluations of many sorts. Germline variations distinguish individuals from each other 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 and can sometimes cause devastating genetic disorders, such as autism (Sebat et al., *Science 316:* 445 [2007]). We have formulated a hypothesis unifying spontaneous and inherited mutation in the etiology of autism (Zhao et al., *Proc Natl Acad Sci 104:* 12831 [2007]). Much of the lab is dedicated to devising methods for data interpretation and building quantitative genetic models.

Cancer and Leukemia

We use copy-number data and DNA-methylation status to study breast cancer (Hicks et al., *Genome Res 16:* 1465 [2006]) and B-cell chronic leukemia (Grubor et al., *Blood 113:* 1294 [2008]). We seek to identify the loci most frequently mutated in cancers and leukemias, and among them to determine which might be causative (Zender et al., *Cell 125:* 1253 [2006]; Xue et al., *Genes Dev 22:* 1439 [2008]; Zender et al., *Cell 135:* 852 [2008]; Bric et al. 2009), and to determine if genomic data can be used to predict the outcome of the disease (Hicks et al., *Genome Res 16:* 1465 [2006]), its response to therapy (McArthur et al. 2009), and the early detection of its recurrence. In addition to assessing the role of copy-number mutation in cancer etiology and outcome, we have developed methods to assess the role of DNA methylation changes (Hodges et al. 2009; Kamalakaran et al. 2009; S Kamalakaran et al., in prep.). A particular emphasis is using copy-number data to assess the population substructure of tumors (Navin et al., *Genome Res 20:* 68 [2010]). We have had success with single-cell genome sequence analysis, a tool that shows extensive promise for applications in clinical and basic cancer biology (N Navin et al., in prep.). All of the above studies are collaborations with scientists at CSHL (Powers, Lowe, McCombie, and Hannon labs) and at other institutions.

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, the role of spontaneous (or de novo) germline CNVs. Our findings established that germline mutation is a more significant risk factor for autism spectrum disorders (ASD) 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. We also study the role of spontaneous mutation in congenital heart disease (a collaboration with Dorothy Warburton at Columbia University), rheumatoid arthritis (with Peter Gregersen at North Shore University Hospital), and pediatric cancers (with Ken Offit at Memorial Sloan-Kettering Cancer Center).

One of the de novo events we identified in autism was a deletion on 16p (Sebat et al., *Science 316:* 445 [2007]). 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, and she has continued to search for phenotypic consequences. 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 the 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. Autism incidence and sibling concurrence rates are consistent with a model in which new or recent mutations with strong penetrance explain the majority of autism in males and are consistent with a one-hit event.

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. This collection is of high-functioning children, and it has a 7:1 bias of males to females. Early initial results confirm our previous findings, and we observe de novo (copy-number) mutation more frequently in children with autism than in their unaffected siblings. The statistical evidence is strong for deletion events, but much weaker for amplifications, an assessment that was not possible before because of lack of statistical power. 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 for 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.

The data deepen the mystery of the male bias in autism. We are developing testable theories, including the possibility that the pattern of monoallelic expression is different in males and females during early development. Regardless of the model, if females require two-hit events, it enables us to estimate the proportion of autism that is one-hit in males. It is (the incidence in males minus the incidence in females) divided by the incidence in males.

Our study based on copy number does not pinpoint the genes that cause autism, because even the narrow events typically contain multiple genes. Pathway analysis, performed in collaboration with Ivan Iossifov at CSHL, does suggest a plausible set of interrelated genes. We are now pursuing our leads by sequence analysis of trios (mother, father, and child) from the Simons collection (a collaboration with the McCombie Lab at CSHL). We are conducting a search for de novo point mutations that disrupt function in our candidate genes. From our unified hypothesis, knowledge of the rate of de novo mutation in the germline, and the rate of autism in males, we estimate that there are on the order of 300–500 autism genes. In the 1000+ trios we expect to sequence, we predict to see a signal in the form of recurrent mutations only in the actual autism genes.

Data Generation, Analysis, and Quantitative Modeling of Genetic Process

A major part of our group's effort centers on the generation, analysis, and interpretation of high-volume data. This includes developing protocols for handling microarray copy-number data, 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; as well as construction of databases so that we can communicate our results to others. Our novel contributions include methods to attenuate system noise in array hybridizations, parameterize 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. We are now also using high-throughput DNA sequence data to measure mutation rates. This entails becoming familiar with and developing new methods for data analysis. Our first "product" in this line is a method to correctly call copy number from the DNA sequence read density of single-cell genomes.

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CANCER: SIGNAL TRANSDUCTION

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

Mikala Egeblad and colleagues study tumors and in particular the microenvironment in which tumors arise and live. Solid tumors are abnormally organized tissues that contain cancer cells, stromal cells, and the extracellular matrix. Communications between the different components of the tumor influence its progression, for example, by regulating metastasis, the immune response to tumors, or the response to therapy. The lab seeks to separate functions and behaviors of the different stromal components of tumors, using mouse models of breast cancer and real-time imaging of cells in tumors in live mice. This enables them to follow the behaviors of and the interactions between cancer and stromal cells in tumors during progression or treatment. Among the tumor-associated stromal cells, their main focus is on myeloid-derived immune cells, a diverse group of cells that can enhance angiogenesis and metastasis and suppress the immune response against tumors. Egeblad is interested in how different types of myeloid cells are recruited to tumors and how their behavior in the tumor microenvironment, for example, their physical interactions with cancer cells and other immune cells, influence cancer progression. Stromal cells and the extracellular matrix can also influence drug responses, for example, by impairing drug delivery to the cancer cells. The lab is addressing how therapy affects cancer and stromal cells in different tumor microenvironments, in part by using imaging to follow the response to chemotherapy in mice in real time.

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 have unique sets of chromosomes and some can produce aggressive epithelial cancers in mice. Having developed a method of producing hybrid cells more efficiently—a means of isolating viral fusogenic proteins—Lazebnik and colleagues are attempting to induce fusion under controlled conditions in order to explore the consequences 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 thinking about this aspect of cancer biology. Using sophisticated model systems such as three-dimensional cell culture platforms and transgenic mice, his team found that a protein called Scribble normally regulates proper differentiation of breast epithelial cells and coerces them into the correct organization and shape and enforces resistance to cancer. They also found that deregulation of the Scribble pathway results in the development of undifferentiated tumors in mice. Muthuswamy's team finds that Scribble is frequently mislocalized from cell membranes or not expressed in human breast cancer lesions, which suggests that understanding the pathways regulated by Scribble can help to identify therapies aimed at preventing precancerous lesions from becoming invasive.

Darryl Pappin's lab develops chemical and computational methods for analysis of proteins and peptides. These are fundamental tools for proteomics and are vital in 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. Skowronski and colleagues have discovered new details about how a simian strain of the AIDS virus replicates in macrophages, a type of immune-system cell. Their studies have revealed that 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 its 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 mutation in the EGF (epidermal growth factor) receptor 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 a 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, which 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. They have, for instance, sought to define the role of JNK stimulatory phosphatase 1 in regulating a signaling pathway critical in Parkinson's and have investigated new roles for PTPs in regulating signaling events in breast cancer, identifying three PTPs as potential novel tumor suppressors. This year, Tonks and colleagues published results of experiments suggesting one way in which cascades of intracellular signals are regulated at what they call a decision point, where cells commit to repair broken DNA strands or commit suicide following DNA damage. A protein, EYA, was found by the lab to regulate the formation of specialized microenvironments on DNA called γ-H2A.X foci, which allow the cell to summon repair enzymes to the site of broken DNA strands.

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 tumors to develop in animal models of prostate cancer. His team later found that complete loss of PTEN paradoxically triggers senescence, an arrested state that delays or blocks cancer development in affected cells. These findings explained why many patients only display partial loss of this tumor suppressor and establish a novel mechanism of cancer initiation. His lab has recently been expanding these findings in collaboration with clinicians at Memorial Sloan-Kettering Cancer Center, with the aim of identifying patients who have developed tumors with metastasis-favoring mutations. The interdisciplinary team aims to generate mouse models that accurately reflect the core genetic changes of human metastatic prostate cancer to develop novel therapies using small molecules or RNA interference technology developed at CSHL. In addition, Trotman's lab is studying regulation of PTEN stability and nuclear transport, as many patients have tumors that aberrantly target PTEN for cytoplasmic degradation.

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 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-linked gene called *oligophrenin-1* (*OPHN1*), part of an effort to connect the genetic abnormalities associated with mental retardation to biological processes that establish and modify the function of neuronal circuits. They discovered that spontaneous activity drives the protein OPHN1 into dendritic spines, where they stabilize synaptic AMPA receptors. They showed how defective OPHN1 signaling results in loss of spines, synaptic strength, and plasticity. They also studied what happens on the presynaptic side of the synapse, discovering that OPHN1 helps neurons transmit messages by controlling the retrieval of synaptic vesicles. This suggests that the symptoms of X-linked mental retardation could stem not only from defects in dendritic spine structure and function, but also from inefficient synaptic vesicle retrieval.

INTERACTIONS BETWEEN TUMOR AND HOST IN CANCER

M. Egeblad E. Nakasone
P. Sinha

Cells and the extracellular matrix (ECM) form tissues, and collections of the tissues join together in structural and functional units to form organs. Solid tumors are not random mixtures of cells and ECM, but rather, they resemble organs, although structurally and functionally abnormal. Organs are composed of the cells that perform the main organ function (e.g., secrete hormones or enzymes) and the stroma, the supportive framework of an organ. The stroma can be divided into several classes: the ECM, which is composed of proteoglycans, hyaluronic acid, and fibrous proteins (e.g., collagen), and stromal cells. The stromal cells include mesenchymal supporting cells (e.g., fibroblasts and adipocytes), cells of the vascular system, and cells of the immune system. Interactions between epithelium and stroma are essential for normal organ development as well as for tumors. As tumors develop and progress, they undergo dramatic morphological changes, which involve both the cancer cells and the stroma. Stromal components proposed to have a more pronounced tumor-promoting function in advanced stages than in earlier stages include the immune cell infiltrate and type I collagen.

We have developed an imaging method that permits real-time spinning disk microscopy of tumor–stroma interactions in mouse models of human breast cancer. This allows us to follow, for example, vascular leakage, immune cell infiltration, and cancer cell proliferation and death in real-time. We use this technology to understand how breast and pancreatic cancer is affected by the stroma.

Effects of the Tumor Microenvironment on Response to Cancer Therapy

Despite the large number of patients treated with cytotoxic chemotherapy, we are literally blind to the effects of the treatment on cancer cells in intact tumors. Knowledge of the mechanism of actions of chemotherapy mostly comes from cell culture experiments or xenograft animal models. Such models do not reflect the complex interactions between cancer cells and their microenvironment found in spontaneous tumors. Mutations in cancer cells can result in anticancer drug resistance. However, the abnormal organization of the tumor tissue affects the ability of anticancer drugs to reach the cancer cells. In normal tissues, cells are within a few cell diameters of a blood capillary, enabling efficient drug delivery. When cancer cells proliferate faster than the cells that form capillaries, the resulting increase in distance from blood vessels impairs drug delivery. The tumor vasculature is often poorly organized with leaky vasculature and sparse or absent lymphatics. This leads to increased interstitial fluid pressure, thereby inhibiting drug distribution. The rate of diffusion through the tumor tissue is also affected by the properties of drugs (e.g., molecular weight, shape, charge, and solubility), by uptake in cancer or stromal cells, and by binding to the ECM (Fig. 1). For example, type I collagen influences the distribution of antibodies and chemotherapeutic drugs.

We are using our imaging technology to study how the microenvironment influences the response to cytotoxic therapy, focusing on drug distribution. Together with Dr. Lisa Coussens' Laboratory at the University of California, San Francisco, we have shown that vessel stability in tissues involves matrix metalloproteinase activity and transforming growth factor-β1 (TGF-β1). Following acute tissue damage, plasma proteins rapidly extravasate out of the vasculature in wild-type mice. Short-term treatment of mice in vivo with a broad-spectrum metalloproteinase inhibitor or an activin-like kinase 5 (ALK5) inhibitor further enhanced vessel leakage. Neoplastic tissues are in a constant state of tissue damage and exhibit hyperleaky vasculature. Using imaging, we have found that inhibition of ALK5 or knock-out of matrix metalloproteinase-9 (MMP-9) in mammary carcinomas further enhance vascular leakage (Fig. 2). We are currently testing if this increased leakage can be used to improve the delivery of therapeutics into tumors.

The microenvironment can regulate anticancer drug responses, but we are also finding that drug treatment can change the microenvironment of tumors. We are focusing on the effects of cytotoxic therapy on the innate immune cells of the myeloid lineage. Breast carci-

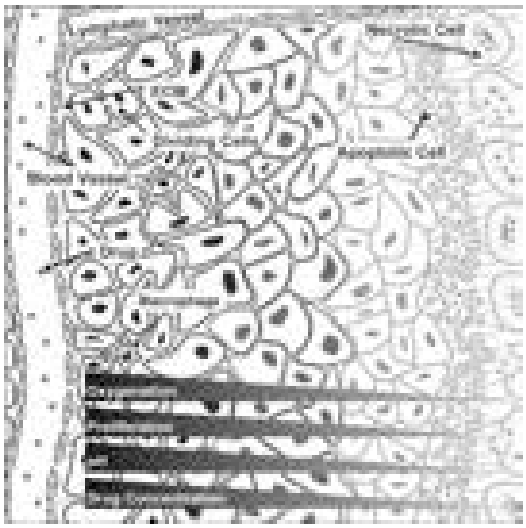

Figure 1. Tumor tissue organization influences drug response. The overall architecture of the tumor has a direct effect on the ability of a cancer drug to penetrate the tissue and reach the cancer cells. Abnormal leakage from blood vessels, together with insufficient lymphatic drainage, especially from the middle of tumors, contributes to increased interstitial pressure in the tumor tissue that inhibits penetration of drugs into the deeper areas of the tumors. Cancer drug penetration is also limited by their binding to ECM proteins, such as collagen, or their uptake by stromal cells, such as macrophages. Cancer cells furthest away from the blood vessel not only are exposed to the lowest drug concentrations, but also receive the lowest amounts of nutrients and oxygen from the circulation and therefore have the lowest proliferative index. Because many cancer-cell drugs preferentially target actively proliferating cells, this effect contributes to the inability of drugs to target cells in hypoxic areas. Cancer cells further from the blood vessel are also exposed to a low-pH microenvironment where many cytotoxic drugs become inactive. Thus, the organization of the tumor tissue results in limited drug availability and efficacy in the hypoxic areas of tumors, areas speculated to contain some of the most aggressive cancer cells. The changing microenvironment in different parts of the tumors therefore results in an apparent difference in drug sensitivity.

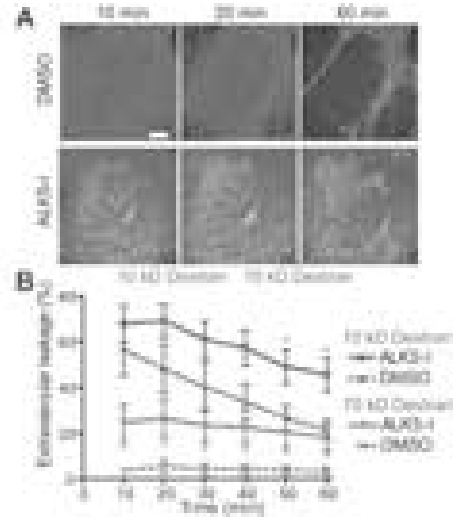

Figure 2. Regulation of drug distribution in tumors by TGF-β receptor signaling. (*A*) Inhibition of TGF-β receptor signaling (ALK5-I) increases the infiltration of dextran into mammary carcinomas compared to control treated mice (dimethylsulfoxide). Live mice were imaged for 60 min after intravenous (i.v.) injection of fluorescent dextrans and quantum dots (Qdots). Bar, 100 m. (*B*) Quantification of the increased dextran infiltration into late-stage carcinomas after inhibition of TGF-β receptor signaling (ALK5-I, $n = 5$ mice per treatment).

nomas are infiltrated with different types of myeloid cells, including monocytes, neutrophils, and different types of macrophages with either tumor-promoting or -inhibiting functions. The myeloid cells can promote tumor progression by stimulating cancer cell proliferation, tumor angiogenesis, and metastasis or by suppressing the immune response. A common side effect of chemotherapeutic treatment is the inhibition or killing of myeloid cells in the bone marrow. By spinning disk confocal microscopy, we have observed that myeloid cells are recruited to tumors treated with cytotoxic chemotherapy. These myeloid cells express neutrophil and monocyte markers, and tumor lysate from treated mice contains increased levels of the chemokine CCL2, which is involved in recruitment of monocytes and neutrophils. We are currently investigating whether the changed myeloid cell infiltrates influence the overall tumor response to chemotherapy.

Collagen Architecture in Cancer Progression

The interstitial matrix is a major category of ECM and consists of macromolecules such as fibrillar collagens, fibronectin, and proteoglycans. Type I collagen is the major fibrillar collagen in tissues, and it forms a scaffold that provides stability to tissues. Type I collagen also has signaling functions mediated by integrins and discoidin domain receptors. The synthesis and proteolytic remodeling of the fibrillar type I collagen increases in many tumors. Type I collagen-degrading enzymes, including matrix metalloproteinases (MMPs), are implicated in cancer progression. MMP-dependent collagen remodeling promotes cancer progression by creating spaces for cells to migrate and by producing fragments of collagen with new biological activities.

By genetic targeting of both type I collagen and the MMP that cleaves it, we have found that cleavage of col-

lagen by stromally produced MMPs controls ductal epithelial invasion in normal mammary gland development in mice. Epithelial ductal invasion is reduced in the absence of MMP14 (MT1-MMP) or MMP2 or when collagen is rendered resistant to MMP cleavage by mutation. To address the role of collagen remodeling for epithelial invasion in cancer, we have cross-bred mouse mammary tumor virus–polyomavirus middle T antigen (MMTV-PyVmT) mice, which develop aggressive mammary carcinoma, with mice with MMP-resistant collagen. Primary tumor size was not affected, but metastasis to the lungs was significantly reduced.

Tumors are often discovered as nodules that are harder than the surrounding tissue. This reflects the changes in ECM stiffening and architecture. An abnormal collagen architectural structure correlates with tumor progression. Whereas collagen fibers are curly and oriented in parallel to normal or hyperplastic epithelium, there is a progressive change in the fibers so that they are straighter and mostly perpendicular to the tumor border in the late stages. This changed architecture may promote cell invasion by enabling cells to migrate along the collagen fibers or by enhancing integrin signaling. Enzymes of the lysyl oxidase family, involved in collagen fiber cross-linking, promote cancer progression and metastasis. In collaboration with Dr. Weaver's lab at the University of California, San Francisco, we have found that inhibiting collagen cross-linking in mouse models of mammary carcinoma changes tumor initiation and progression: Tumor onset is delayed and the progression stage of the tumors is lower. Tumor fibrosis and cancer cell phosphoinositol-3 kinase (PI3K) activity were also reduced. We are now investigating the effects of collagen cross-linking in pancreatic carcinoma.

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M. Egeblad

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 viruses can contribute to carcinogenesis and/or tumor progression by fusing cells, thereby combining properties of diverse cells and further increasing their diversity through inducing chromosomal instability.

To test the possibility that fusion of tumor cells to hematopoietic cells can facilitate metastasis of cancer cells, we began a collaboration with Dr. James Bliska (Stony Brook University), who provided us with mouse macrophage progenitors and taught us how to culture them. We developed an approach to derive clonogenic hybrids of these cells with human prostate cancer and will test these hybrids for their metastatic potential.

To understand how cell fusion causes massive chromosomal instability, a phenomenon that we reported previously, we established a collaboration with Dr. Keith Killian (National Institutes of Health) to analyze early genomic and epigenomic changes occurring in hybrids.

Overall, by comparing the properties of cell hybrids with those of cancer cells, we hope to better understand carcinogenesis and tumor progression.

EPITHELIAL CELL BIOLOGY AND CANCER

S. Muthuswamy D. Akshinthala, V. Aranda, M. Feigin, K. Krishnamurthy, A. Lucs, A. Rosenberg, B. Xue, L. Zhan

The molecular mechanisms that regulate the development and progression of premalignant lesions are poorly understood. A better understanding will aid in identification of new predictive biomarkers and drug targets for premalignant disease. Today, pathologists use a number of criteria to diagnose and predict prognosis of premalignant breast lesions and 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 differentiation. We believe that gaining a better fundamental insight into the mechanisms that regulate changes in cell differentiation during the development and progression of premalignant lesions will identify the molecular targets that pathologists can use tomorrow to make a better prognosis of premalignant lesions. In this process, we may also identify novel strategies to treat premalignant 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 is referred to as basolateral surface. This characteristic organization is lost early in premalignant disease and the tissue continues to lose 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 current understanding of the molecular mechanisms by which epithelial cells establish and maintain this asymmetry, also referred to as polarity, suggests that there are several proteins and protein complexes which function as a highly interactive network during establishment and maintenance of epithelial cell polarity. One such complex, the Par6/aPKC/Cdc42, regulates polarization processes during epithelial morphogenesis, astrocyte migration, and axon specification. We have identified a direct interaction between ErbB2 and the Par complex that is necessary for ErbB2-induced transformation of organized mammary epithelial cells. This is a major discovery because it identifies, for the first time, that oncogenes can directly interact with polarity protein complexes. We are currently dissecting the signaling pathway by which ErbB2 disrupts the normal function of the Par complex and how it affects the activity of the polarity kinase aPKC (atypical protein kinase C), as well as the downstream effects on other components of the Par6/aPKC pathway. The evidence we have accumulated over this year points to a role for the aPKC target, Lgl, as a critical mediator of ErbB2-induced disruption of cell and tissue architecture. We are currently investigating the role this pathway has during ErbB2-induced transformation of cells as well as the role it has during progression of premalignant breast lesions in human breast.

In addition to interacting with Par6/aPKC, activation of ErbB2 also induces disruption of Par3 from the polarity complex. During the last year, we asked if Par3 has a role during ErbB2-induced transformation of human mammary epithelial cells. We find that Par3 maintains the integrity of cell–cell junctions and inhibition of cell invasion, suggesting that Par3 may have an important role during progression of ErbB2-positive breast cancers. We are currently investigating how Par3 regulates ErbB2-induced disruption of 3D acini and its implications for ErbB2-positive human breast cancers.

We are interested in investigating whether changes in polarity proteins function as drivers of tumorigenesis. To directly test this possibility in mouse models of human breast cancer, we have generated and characterized a mouse model that expresses an altered version of

a polarity protein, Scribble. These mice develop tumors, albeit after a long time and with low frequency. These observations are striking because it demonstrates that alterations in polarity proteins can function as initiators of tumorigenesis. These observations also suggest that alterations in polarity proteins are likely to make the cells and tissue receptive for random somatic mutations that drive the tumorigenesis process. We are currently investigating the mechanisms by which these tumors arise, and in doing so, we hope to identify molecular alterations that can function as predictive biomarkers for preneoplastic lesions.

In addition to regulating apical–basal polarity in epithelial cells, polarity proteins are also critical regulators of normal differentiation of progenitor cells in *Drosophila*. We have discovered that deregulation of Scribble in pluripotent mammary cells promotes symmetric cell division and blocks differentiation of progenitor cells along a luminal epithelial lineage. We find that loss of Scribble activates Notch signaling. Inhibition of Notch signaling rescues the loss of the differentiation phenotype, demonstrating that Scribble regulates differentiation by controlling Notch signaling. These observations identify a novel relationship between cell polarity pathways and normal differentiation of mammary progenitor cells. Because changes in pathways that regulate normal development are implicated in initiation of cancer, we believe that understanding the mechanisms by which Scribble regulates epithelial differentiation will have important implications for development of premalignant disease.

In addition to investigating cell polarity pathways and biology of premaligant disease, we also are interested in developing a deeper understanding of pathways downstream from ErbB2 signaling that are required for transformation of human mammary epithelial cells. Because patients with ErbB2-positive cancers fare poorly in the clinic, we believe that this will identify novel strategies for therapeutic intervention of ErbB2-positive cancers. In this context, we had previously reported the identification of Brk, a Src family tyrosine kinase, as a novel member of the ErbB2 signaling pathway and a potential drug target for ErbB2-positive breast cancers. During the past year, we have completed a study that was aimed at developing a deep understanding of the receptor-proximal events that regulate transformation of epithelial cells. Using inducible activation of ErbB2 autophosphorylation site mutants and an MCF-10A three-dimensional culture system, we investigated pathways used by ErbB2 to transform epithelia. We reported that ErbB2 induces cell proliferation and loss of three-dimensional organization by redundant mechanisms, whereas it disrupts apical basal polarity and inhibits apoptosis using Tyr-1201 and Tyr-1226/7, respectively. Signals downstream from Tyr-1226/7 were also sufficient to confer paclitaxel resistance. The Tyr-1226/7 binds Shc, and knockdown of Shc blocked the ability of ErbB2 to inhibit apoptosis and mediate paclitaxel resistance. Tyr-1226/7 is known to activate the Ras/Erk pathway; however, paclitaxel resistance did not correlate with activation of Erk or Akt, suggesting the presence of a novel mechanism. Thus, we demonstrated that targeting pathways used by ErbB2 to inhibit cell death is a better option than targeting cell proliferation pathways. Furthermore, we identified a novel function for Shc as a regulator of apoptosis and drug resistance in human mammary epithelial cells transformed by ErbB2. Thus, research in our laboratory is developing a better understanding of how cell polarity pathways are regulating cell transformation and how ErbB2 transforms human breast epithelial cells. Together, these studies are likely to identify new ways for treating breast cancer.

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PROTEOMICS LABORATORY AND PROTEOMICS SHARED RESOURCE

D. Pappin C. Fu
S. Peacock
C. Ruse

The year 2009 was one for restructuring and rebuilding. The proteomics laboratory had been effectively mothballed from the fall of 2007, with CSHL researchers looking to outside resources to fulfill protein and peptide analysis. I was recruited in late 2008 to a faculty position, with scientific oversight for the proteomics Shared Resource. It was decided early on that the laboratory should remain in the same location, in the Beckman lower level, but it was soon realized that this would require complete refurbishment of the existing laboratory space. Planning was completed by the end of the year, and searches were started to recruit additional laboratory members. In March, Dr. Cristian Ruse joined the lab from Scripps (La Jolla) as manager of the Shared Resource facility. Some laboratory space was quickly refurbished by the beginning of April while building work continued on the main laboratory across the hall. Two of the smaller mass spectrometers (an existing LTQ ion trap and a new Agilent 6520 QTOF) were installed and commissioned, and some small-scale projects were undertaken. In came equipment for packing homemade capillary LCs, a brace of Proxeon Capillary HPLC pumps, and database searching software was installed on the CSHL bluehelix cluster (with much help from Todd Heywood and other members of IT). July saw the arrival of Cexiong Fu (from University of Medicine and Dentistry, New Jersey), just in time to provide essential support for the 2009 Proteomics Course, breaking new ground in the recently completed laboratories on the Hillside campus. In September, the main laboratory space was completed and the existing two instruments were moved and joined in October by a Vantage triple-quadrupole mass spectrometer, intended to be used primarily for multiple reaction monitoring (MRM) work. Instrument installations were completed in early December with the arrival of the Thermo Orbitrap XL mass spectrometer, with the first two-dimensional MudPIT samples being run just in time for Christmas. Samantha Peacock joined the lab immediately after the New Year.

A considerable amount of time during the fall was taken up in fitting out and testing the new instruments. An expanding repertoire of techniques was and continues to be added to instruments and taught to individual lab members. These techniques now include quantitative two-dimensional whole-proteome analysis using iTRAQ or SILAC on the ion traps, phosphopeptide site identification in proteins and protein complexes, and tailored data analysis. Efforts are currently being directed to optimizing iTRAQ quantitation using the Orbitrap and high-energy collision dissociation (HCD) and phosphopeptide enrichment protocols using titanium dioxide columns. By late December, a number of facility projects were under way.

Phosphorylation of Subunits of the Origin Recognition Complex

For this investigation, we established two independent procedures for the analysis of site-specific phosphorylation from the origin recognition complex (ORC) protein complexes. First, we implemented a *variant* of multidimensional protein-identification technology (MudPIT) for enhanced detection of phosphopeptides. Our method harnesses the chromatographic properties of different packing materials for MudPIT columns. The approach begins to segregate phosphopeptides and normal peptides in different fractions of the MudPIT process, where phosphopeptides elute at low salt and are concentrated in a few early chromatographic fractions. Phosphopeptide mapping was performed with multiple proteases, both specific and nonspecific, to produce redundant overlapping sets of peptides, before analysis by liquid chromatography mass spectrometry (LCMS). In a complementary approach, we also enriched phosphopeptides with titanium dioxide affininity chromatography. In total, we identified 24 phosphorylation sites, of which 16 were site-localized based on MS/MS fragmentation and eight were unlocalized. For this purpose, we used the InSpecT software toolkit that allows for scoring of phosphate localization following statistical identification of nontryptic phosphopeptides.

MudPIT Analyses of SF2 Complexes

We completed a large-scale project that investigated interaction partners of the SF2/ASF oncoprotein and other splicing factors. To this end, we implemented an efficient MudPIT strategy based on autosampler delivery of salt pulses. By combining salt pulses with organic solvent gradients, we were able to run back-to-back MudPIT analyses with virtually no carryover. In this format, we were able to complete more than 30 12-step MudPIT analyses in a brief period of time. Computational investigation of data acquired for this large project necessitated parallelized database searches. The MASCOT search program is currently running on 64 processors allowing for fast processing of large batches of MudPIT runs. To optimize our MudPIT system, we also developed a new routine for data-dependent acquisition on the linear ion trap (LTQ). In this case, the LTQ was set to acquire peptide masses with higher resolution by scanning in an enhanced scan mode. Both selection of peptide precursors for further CID fragmentation and database searches with MASCOT benefited significantly from the higher resolution and charge state assignment.

C. Ruse

CELL SIGNALING IN HIV PATHOGENESIS

J. Skowronski M. Gierszewska
K. Hrecka

Our interest lies in understanding the molecular mechanisms that underlie the pathogenesis of AIDS and, in particular, in 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 accessory virulence factors of human and simian immunodeficiency viruses (HIV and SIV). We also initiated a new direction aimed at understanding the biochemical underpinnings of selected innate mechanisms that block HIV infection, such as that mediated by cellular tripartite motif-containing protein 5 (TRIM5). Accordingly, we have directed a major effort toward the identification of mechanisms and downstream effectors that mediate the effects of Vpr and Vpx in the infected cells. These experiments have led to the description of several novel cellular proteins that tightly associate with these factors in hematopoietic cells. Among them are the E3 ubiquitin ligase complexes that regulate cell cycle progression and the repair of damaged DNA. 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. Our current effort is concentrated on the identification of cellular proteins that are recruited to these E3 complexes by Vpr and Vpx, as they are likely cellular factors that modulate HIV replication. Here, we describe in more detail our studies of lentival Vpr and Vpx.

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 *vpr* 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 they 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 has 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 Usurp a Specific Cullin-4-based E3 Ubiquitin Ligase Using a DCAF1 Substrate Receptor

We used a combination of biochemical and proteomic approaches to identify cellular proteins associated with human and simian Vpx/Vpr proteins. With these tools, we previously showed that Vpr and Vpx accessory factors target and function through Cullin-4-based E3 ubiquitin ligase using a DCAF1 substrate receptor. Our findings support a model in which Vpr, as well as Vpx, usurps Cullin-4 E3 ubiquitin ligase using the VprBP/ DCAF1 to inactivate a cellular factor that inhibits lentivirus replication in infected cells. 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 at identifying cellular proteins whose ubiquitination is altered by Vpr and Vpx. We expect that our findings will advance the understanding of these important virulence factors.

TRIM5 Is Bound to the 19S Regulatory Particle of the 26S Proteasome

TRIM5 is a cellular protein that can block infection of HIV-1 as well as other retroviruses. TRIM5 proteins restrict the retrovirus life cycle at least at two stages: by tar-

geting early postentry events that lead to reverse transcription of the viral genome, which requires functional 26S proteasome, and by interfering with transport of the viral preintegration complex to the nucleus. To address the underlying mechanisms, we performed comprehensive proteomic analyses of TRIM5-associated cellular proteins from cell lines stably expressing rhesus monkey TRIM5. These experiments were performed in close collaboration with Michael Washburn, Laurence Florens, and Selene Swanson at the Stowers Institute for Medical Research (Kansas City). Strikingly, we identified 26 out of 31 subunits of 26S proteasome in highly purified TRIM5 preparations. This demonstrated that TRIM5 tightly associates with 26S complex, but it was surprising in that the 26S complex normally dissociates into its 19S regulatory and 20S catalytic subcomplexes under the purification conditions used. This probably reflects TRIM5 degradation because such a behavior is expected of stalled 26S proteasome–substrate complexes. We next addressed the roles of TRIM5 polyubiquitylation. A systematic scan of the TRIM5 molecule with lysine-to-arginine substitutions revealed that at most only four lysines located in the SPRY domain are required for TRIM5 to restrict infection, and these lysines are not the sites of polyubiquitylation. We also analyzed the ability of a mutant TRIM5 molecule (K74R) that retains only six lysines in its RING domain and is not polyubiquitylated to bind 26S proteasome. TRIM5 K74R stably and specifically associated with 26S proteasome, as it precipitated the complete 26S proteasome complex under conditions that stabilize the 26S complex. Strikingly, and in contrast to wild-type TRIM5, K74R precipitated only the 19S regulatory subcomplex under conditions that favor 19S dissociation from 20S, thus indicating that it docks specifically to the 19S regulatory subcomplex. The spectrum of 19S subunits found to be associated with K74R suggests that it docks to 19S probably via its base, similar to other proteins that modulate proteasome function, and, importantly, confirms that K74R binding to 19S is not mediated by TRIM5 polyubiquitylation, because the 19S subunits that serve as polyubiquitin receptors were not found. Our findings indicate that TRIM5, besides being degraded by the proteasome, also stably associates with the 19S regulatory subcomplex by a polyubiquitylation-independent mechanism. We hypothesize that via its binding to the 19S subcomplex TRIM5 recruits the 26S proteasome to the restricted viral capsid and that this physical interaction with the 19S subcomplex is crucial for TRIM5's full ability to restrict retrovirus infection.

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

R. Sordella M. Camiolo M. Pineda
S. Fenoglio A. Saborowski
T. Lindsted Z. Yao

The goal of our lab is to identify better cancer therapies. Despite the large variety of genetic abnormalities that they typically harbor, cancer cells are incredibly sensitive to the reversal of certain mutations. A few years ago, Bernard Weinstein described this phenomenon as "oncogene addiction." Examples are provided by c-Myc, Ki-Ras, Ha-Ras, Bcr-Abl, Her-2, and Wnt1. Understanding why cancer cells are dependent on specific oncogenes could lead to the development of more effective therapies. Recently, we identified new epidermal growth factor receptor (EGFR) somatic mutations in non-small-cell lung cancer (NSCLC) patients that respond dramatically to treatment with gefitinib (Iressa) or erlotinib (Tarceva), two selective inhibitors of EGFR. In particular, we demonstrated that the presence of these mutations render the cells "addicted" to signaling activated by the mutant EGFR alleles. This finding spurred new excitement on the possibility of treating cancer patients in a "personalized" manner. Based on our studies in several cancer centers, lung cancer patients are now screened for the presence of EGFR mutations, and based on the genetic status of the tumors, patients are treated either with standard chemotherapy or with EGFR inhibitors.

TGF-β1 and TGF-β2 Are Necessary and Sufficient for Erlotinib Resistance and EMT and, through an Increased Activation of the IL-6 Axis, to Unleash Tumor Cells from their EGFR Dependency

Unfortunately, in a majority of cases, the dramatic responses observed upon treatment with EGFR inhibitors are transient, and within a short period of time, patients who initially responded to treatment suffer a relapse. Studies have now shown that the acquisition of an additional mutation in exon 20 of EGFR, resulting in a threonine–methionine substitution at position 790 (e.g., T790M mutation) and/or amplification of c-MET, account for ~50% of cases of erlotinib-acquired resistance.

To uncover new molecular mechanisms of gefitinib and erlotinib resistance in NSCLC, we have developed a cell-based system. We grew lung-tumor-derived cells that express EGFR oncogenic mutations in the presence of a high concentration of Tarceva, and we selected cells that become unleashed from their EGFR dependency. Interestingly, ~13% of these cells displayed morphological features of mesenchymal cells. They not only lost their cobblestone-like morphology and acquired a more elongated shape, but they also displayed enhanced motility and invasion capabilities. The epithelial-mesenchymal transition (EMT) is a complex program that involves activation of many different signal transduction pathways. Among them, we were able to show that an increased autocrine secretion of transformation growth factor-β (TGF-β1) and TGF-β2 was sufficient to induce the expression of master regulators of EMT such as SNAI and ZEB2 and of interleukin 6 (IL-6). Interestingly, we found that an augmented secretion of IL-6 was sufficient to decrease the cells' sensitivity to erlotinib treatment.

Our studies thus present first evidence that TGF-β1 and TGF-β2, in addition to having pivotal roles in the EMT, are also important determinants of primary erlotinib resistance in NSCLC through the establishment of an EGFR-independent autocrine secretion of IL-6.

Whereas our data indicate that a cell-autonomous mechanism was responsible for the increased expression of TGF-β1 and TGF-β2, these same cues may also be produced by the tumor microenvironment, thus inducing erlotinib resistance in a paracrine fashion. Interestingly, IL-6 is a pleiotropic cytokine that exerts important biological functions in a wide variety of systems and processes, including inflammation, acute-phase reaction, nervous and endocrine systems, bone metabolism, and hematopoiesis. Thus, we hypothesized that the secretion of IL-6 by the tumor microenvironment during the course of inflammation could impair cell sensitivity to erlotinib. If correct, this hypothesis could provide a new clue to explain the high degree of heterogeneity in the response to erlotinib treatment of NSCLC tumors harboring mutant EGFR. To test this hypothesis, we used a

mouse model system previously described by Vasunia et al. (*Carcinogenesis 15:* 1723–1727 [1994]) in which cutaneous inflammation and secretion of IL-6 are induced by topical treatment of the mouse epidermis with a low dose of 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA).

Specifically, we injected subcutaneously immunocompromised mice with NSCLC cells expressing mutant EGFR. When the tumors reached a volume of ~100 mm^3, we topically treated the epidermis with TPA for 5 days. Mice were then treated with erlotinib for 9 days at a dosage equivalent to that used in patients.

Immunostaining of tumor sections using an anti-IL-6 antibody confirmed that TPA augmented IL-6 expression in the tumors. By comparing tumor burden after different treatments, we found that TPA treatment dramatically reduced the response to erlotinib treatment. To determine whether increased IL-6 produced by the tumor microenvironment upon TPA treatment was responsible for the diminished erlotinib response, we decreased IL-6 availability by injecting the mice with an antibody that neutralizes mouse IL-6 antibody. Remarkably, cotreatment with a mouse IL-6 neutralizing antibody decreased the effect of TPA and increased the tumor sensitivity to erlotinib. These results thus suggest that, in addition to the tumor genome/epigenome (e.g., different genomic or epigenetic aberrations in addition to mutant EGFR such as the T790M mutation or c-Met amplification), differences in IL-6 levels due either to tumor cell-autonomous mechanisms or to the activation of the tumor microenvironment (i.e., inflammation consequent to chemotherapy, infection, or tumor location) may account for differences observed in the response of NSCLC tumors to erlotinib treatment. Several studies have reported increased levels of IL-6 in ~30% of NSCLC. Taken together, these results suggest the intriguing possibility that adjuvant treatments designed to either control inflammation and/or decrease the bioavailability of IL-6 may provide an effective means to improve responses to EGFR TKI treatment in patients with NSCLCs harboring EGFR oncogenic mutations.

Erlotinib-resistant Mesenchymal-like Cells are Present in Cell Lines and in Tumors before Erlotinib Treatment

Several groups recently described the existence of a subpopulation of cells in certain breast-cancer-derived cell lines and in breast cancers that displayed stem-cell-like properties. These cells displayed an increased expression of the surface marker CD44 and a decreased expression of CD24. Significantly, these same cells have mesenchymal-like features, increased migratory and invasive abilities, and augmented metastatic potential compared to the bulk cell population. Because gene expression profile analysis revealed that CD44 expression is higher and CD24 expression is lower in the erlotinib-resistant cells that we generated when compared to the parental cells, we reasoned that in our experimental model system, erlotinib-resistant mesenchymal-like cells could exist as a side population and as such could have been present in the parental-sensitive H1650 cell line before erlotinib treatment.

By using CD44 and CD24 as surface markers, we were able to identify in erlotinib-sensitive parental cells, before erlotinib treatment, a subpopulation of cells with features similar to those of erlotinib-resistant derived cells. Specifically, we found that in the erlotinib-naïve cells, a $CD44^{high}/CD24^{low}$-enriched cell fraction was characterized by a mesenchymal appearance, by increased expression of TGF-β1, TGF-β2, and IL-6. Importantly, these cells were also more resistant to erlotinib treatment compared to the $CD44^{low}/CD24^{high}$ fraction. To exclude the possibility that the occurrence of erlotinib-resistant mesenchymal-like cells was due to artifical in vitro growth conditions and/or to events that occurred during establishment of the cell lines, we extended our analysis to cells obtained from patients with NSCLC. We were able to identify $CD44^{high}/CD24^{low}$ cells in preparations from early-stage erlotinib-naïve human NSCLC tumors and bone marrow of patients with NSCLC. The $CD44^{high}/CD24^{low}$ cells, like the in-vitro-derived erlotinib-resistant cells, had decreased expression of E-cadherin and higher levels of expression of IL-6, Vimentin, TGF-β1, and TGF-β2.

The presence of cells with features similar to those of erlotinib-resistant cells (i.e., heightened expression of TGF-β1, TGF-β2, and IL-6 present in early-stage erlotinib-naïve NSCLC cells from patients) in early-stage erlotinib-naïve tumors suggests that cells which have the potential to be resistant to erlotinib and to display an increased metastatic capability are already present in tumors before erlotinib treatment and therefore could contribute to minimal residual disease. As a consequence, our results indicate that therapies based solely on the inhibition of the EGFR will not be sufficient for the effective treatment of NSCLC patients.

Protein Tyrosine Phosphatases and the Control of Signal Transduction

N.K. Tonks G. Bencze, B. Boivin, F. Chaudhary, X. Cheng, G. Fang, A. Haque, N. Krishnan, L. Li, G. Lin, M. Ramesh, U. Schwertassek, M. Yang, X.C. Zhang

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 protein tyrosine phosphatases (PTPs), which, like the kinases, 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.

During the last year, Gaofeng Fang joined the lab as a postdoctoral fellow, having completed his Ph.D. in the lab of Celine Gelinas at the University of Medicine and Dentistry of New Jersey. In addition, Gyula Bencze joined as a visiting scientist from Mate Hidvegi's lab in Budapest, Hungary.

Currently, there are four broad areas of research in the lab covering functional analysis of members of the PTP family, regulation of PTP function, in particular by reversible oxidation, development of novel approaches to therapeutic intervention in PTP function, and characterization of a knockout mouse model to define the function of JSP1, a member of the PTP family that is a novel regulator of mitogen-activated protein kinase (MAPK) signaling.

Functional Analysis of the "PTPome"

There are ~100 PTP genes in the human genome, and various studies from many labs have pointed to the functional importance of these enzymes in the control of cell signaling. It is now apparent that they have the capacity to act both positively and negatively in the regulation of signal transduction. Furthermore, the PTPs have the potential to display exquisite substrate, and functional, specificity in vivo. Nevertheless, the majority of the members of the PTP family 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. There are several projects in the lab that utilize a library of short hairpin RNAs (shRNAs) that we have generated to target each of the PTPs (five shRNAs for each PTP), which allows us to interrogate systematically the function of these signaling enzymes by RNA interference (RNAi) in various disease models.

The Function of PTPs in Breast Cancer

We have continued our collaborative studies with Senthil Muthuswamy at CSHL in cell models of breast cancer. We have shown that suppression of three PTPs, PTPRG, PTPRR, and PTPN23, enhanced motility of MCF10A mammary epithelial cells. 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 the 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.

The Function of "Missing in Metastasis": A Putative Regulator of RPTPδ

Missing in Metastasis (MIM) is an actin-binding protein that induces actin-rich membrane protrusions, reduces stress fibers, and inhibits cell motility by regulation of cortactin and actin polymerization. Furthermore, expression of MIM is significantly reduced in multiple metastatic cancer cells. Taken together, these observations suggest a function for MIM in suppression of metastasis. MIM has also been shown to bind to a receptor PTP, PTPδ. This interaction may provide a link between tyrosine-phosphorylation-dependent signaling and control of the actin cytoskeleton in metastasis. In collaboration with Rob Lucito and Senthil Muthuswamy here at CSHL, we have been investigating the contribution of MIM to metastasis by studying its function in MCF10A mammary epithelial cell models of breast cancer. We have shown that suppression of MIM by RNAi led to enhanced migration of MCF10A cells. In addition, in MCF10A cells that ectopically express HER2, suppression of MIM cooperated with activation of HER2 to accelerate invasion. Moreover, we observed that the absence of MIM led to a suppression of tyrosine phosphorylation of selected proteins, and this was abrogated by cosuppression of RPTPδ. Currently, we are using mass spectrometry approaches to identify the critical tyrosine-phosphorylated proteins through which MIM and RPTPδ exert these effects.

Functional Analysis of the Protein Phosphatase Activity of PTEN

The tumor suppressor phosphatase PTEN displays intrinsic activity toward both protein and phosphatidylinositol phospholipid substrates in vitro. Whereas the lipid phosphatase activity of PTEN is important for its tumor suppressor function, the significance of its protein phosphatase activity is unknown and represents a gap in our understanding of the function of this important regulator of cell signaling. We have developed an in vivo assay of PTEN function, which has revealed an effect of its protein phosphatase activity on the density of neuronal spines in cultured hippocampal organotypic brain slices. The approach utilized two-photon laser-scanning microscopy and biolistic gene delivery of green fluorescent protein (GFP)-tagged constructs into organotypic slices of the hippocampus. The organotypic slice culture system maintains the tissue architecture of the hippocampus and produces a mosiac that permits analysis of the transfected neuron in a more natural context. This is one of the few systems where images with subcellular resolution can be taken, in real time, from living tissues. In an initial characterization of the enzymatic activity of tumor-derived mutations, we demonstrated that G129E selectively ablates the lipid phosphatase activity of PTEN, leaving protein phosphatase activity intact. Subsequently, a novel Y138L mutant has been shown to preserve the lipid, but not the protein phosphatase activity of PTEN. We have observed that expression of wild-type PTEN in organotypic brain slices led to a decrease in spine density. Strikingly, the G129E tumor-derived, protein-phosphatase-active PTEN mutant produced the same reduction in spine density as the wild-type protein, whereas catalytically dead (C124S) and protein-phosphatase-deficient, but lipid-phosphatase-active (Y138L), mutants were without effect (Fig. 1). PTEN was shown initially to favor acidic substrates in vitro. Interestingly, the carboxy-terminal segment of PTEN contains an acidic stretch of residues that contains multiple sites of phosphorylation by casein kinase 2. We have demonstrated that the PTEN C-tail is a substrate of PTEN protein phosphatase activity in vitro and in 293 cells.

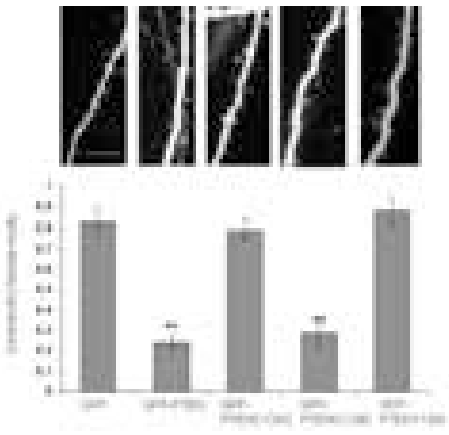

Figure 1. Effects of PTEN expression on spine density in hippocampal neurons. (*Top*) Representative two-photon images of apical dendrites from cells biolistically transfected with GFP-tagged PTEN constructs in organotypic slice cultures of rat hippocampus. (*Bottom*) Quantitation of the effects of each plasmid on spine density. In each case, data were derived from eight different transfected cells in different brain slices and by quantitation of >250 spines.

Furthermore, the dephosphorylation of the PTEN C-tail is required for the reduction in spine density. It has been shown that expression of the C2 domain of PTEN in glioma cell lines, in the absence of the catalytic domain, suppressed migration as efficiently as the wild-type enzyme (Raftopoulou, *Science 303:* 1179 [2004]). It was also shown that dephosphorylation of Thr-383, from a cluster of four phosphorylation sites at the carboxyl terminus of PTEN, was required for inhibition of cell migration by the C2 domain in the context of the full-length protein. Therefore, we tested expression of the isolated C2 domain in our system, but observed no effect on spine density, thereby distinguishing our analysis from the study in glioma cells. In contrast, we have observed that the PDZ-binding motif at the extreme carboxyl terminus of PTEN was required for the reduction in spine density. Our data suggest that the protein phosphatase activity of PTEN and the phosphorylation of Ser and Thr residues in its carboxy-terminal segment are of regulatory significance to the control of spine density, perhaps through controlling interactions between PTEN and PDZ domain-containing proteins. In an interesting symmetry with PI3K, which possesses lipid kinase activity toward exogenous substrates and the ability to autophosphorylate, our data suggest that PTEN may autodephosphorylate; thus, the critical protein substrate for PTEN may be PTEN itself.

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 pKα. 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. We have developed approaches to detect reversible oxidation of PTPs in vivo that involve labeling with biotinylated small molecules the active-site Cys residue in those PTPs that undergo stimulus-induced reversible oxidiation. 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 examining the role of reversible oxidation in regulating PTP function in various cell and animal models.

Development of Novel Approaches to Therapeutic Intervention in PTP Function

Despite the fact that "big pharma" are experts in drug development, they tend to operate only in areas of chemistry in which the individual company is comfortable, coupled with tried and true strategies of generating active-site-directed small-molecule inhibitors. They tend not to commit internal resources to new strategies for therapeutic development, but instead look to the outside for breakthroughs. Venture capitalists now lack the patience for discovery and want a clear path to the clinic that will provide a rapid return on investment, which limits opportunities for novel approaches to drug development in the biotechnology sector. Therein lies the opportunity for academia, with its freedom to think without boundaries and to challenge existing paradigms. In the current economic climate, it is perhaps only in academia that such risks can be taken that are required to develop new strategies that exploit areas and targets currently viewed by industry as "undruggable" and thereby benefit patients through discovery of new therapies. Hopefully, CSHL will make the appropriate investment to seize this historic opportunity and be a catalyst to drive new and creative approaches to drug discovery.

Currently, our work in this area is primarily focused on PTP1B, the prototypic member of the PTP family. 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. In addition, the establishment of PTP1B as a critical positive regulator of signaling downstream from the HER2 oncoprotein tyrosine kinase has identified it as a candidate therapeutic target in breast cancer. As a result, there have been major programs in industry focused on developing small-molecule inhibitors of PTP1B to address these major unmet medical needs. Nevertheless, these efforts have been frustrated by technical challenges arising from the chemical properties of the PTP-active site. Many companies, as well as academic groups, have produced active site-directed inhibitors of PTPs that are potent, specific, and reversible, but their high affinity depends upon their charge, which limits their bioavailability and their potential for drug development. Consequently, new approaches to inhibition of PTP1B, other than active-

site-directed small-molecule inhibitors, are required to reinvigorate drug development efforts.

Harnessing Reversible Oxidation of PTP1B as a Novel Approach to Therapeutic Intervention in Diabetes, Obesity, and Cancer

Previously, we had demonstrated that oxidation of the catalytic Cys residue in PTP1B induced profound changes in the architecture of the enzyme that triggered residues from the active site to adopt solvent exposed positions in a process that was readily reversible. Previously, we had also 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 (reactive oxygen species), would augment the hormone response by removing an inhibitory constraint on signaling.

To achieve this, we have used phage display technology to generate conformation-specific antibodies that recognize oxidation-specific epitopes in the oxidized conformation of PTP1B (PTP1B-OX) that would not be found in the active, reduced enzyme. We generated single-chain fragment-variable (scFv) constructs, which are composed of the variable light (V_L) and variable heavy (V_H) regions of the antibody molecule artificially joined with a neutral peptide linker. We have identified scFvs that bound specifically to the oxidized form of PTP1B in vitro and showed significant inhibition of the reactivation of PTP1B-OX by reducing agent, but did not exert any direct inhibitory effect on the phosphatase activity of the reduced enzyme in assays. In validating this approach further, we observed that expression of the scFvs to PTP1B in cells as "intrabodies" enhanced and extended the time course of insulin-induced phosphorylation of the β-subunit of the insulin receptor and IRS-1, but had little or no impact on the basal level of tyrosyl phosphorylation. Furthermore, expression of the scFv intrabody also enhanced and extended the time course of insulin-induced downstream signaling, measured by phosphorylation of PKB (protein kinase B). These data illustrate that stabilization of the inactive oxidized conformation of PTP1B potentiated insulin signaling in a similar manner to inhibiting the catalytically active form of the phosphatase.

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, then this could provide a new strategy for PTP-directed drug development that may circumvent the difficulties that are faced when targeting the PTP-active site. Currently, we are attempting to initiate high-throughput screening efforts to identify such molecules. Our data illustrate that stabilization of the inactive PTP1B-OX conformation would potentiate insulin signaling in a similar manner to inhibiting the catalytically active form of the enzyme. 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.

Development of Allosteric Inhibitors of PTP1B

Another complementary strategy for development of therapeutics that would avoid targeting the catalytic center of PTP1B would be to look for allosteric inhibitors. We have now identified a novel allosteric site on PTP1B. Originally, we purified PTP1B from human placenta in a 37 kDa truncated form that is stable and readily expressed and purified as a recombinant protein and, therefore, has been the focus of attention to date for biochemical and mechanistic analysis, as well as for drug screening. Nevertheless, PTP1B exists in vivo as a 435-residue protein of ~50 kDa, in which the carboxy-terminal segment, which is deleted from the 37 kDa protein, serves a regulatory function. In the case of TCPTP, the closest relative of PTP1B, we have demonstrated previously that this carboxy-terminal segment is inhibitory to activity. In our hands, the equivalent segment of PTP1B was also inhibitory, but the effects were not as pronounced as in TCPTP.

We have characterized the mechanism of action of a natural product that was shown to induce weight loss,

and increase insulin sensitivity, in diet-induced and genetically obese animal models and was thought to be a noncompetitive inhibitor of PTP1B. We have demonstrated that this molecule was a reversible, selective, and noncompetitive inhibitor of PTP1B. Furthermore, forms of PTP1B that contained the inhibitory carboxy-terminal segment were about tenfold more potently inhibited than the 37-kDa form, which comprises residues 1–321. Sequential deletions from the carboxyl terminus indicated that this high-affinity inhibition was lost when the protein was truncated beyond the segment that exerts an autoinhibitory effect. Consistent with our kinetic analysis, isothermal titration calorimetry (ITC) indicated that there was only one low-affinity site on PTP1B(1–321), but two binding sites on PTP1B(1–394). Scatchard analysis indicated positive cooperativity between the two sites. Binding of inhibitor to the "full-length" enzyme blocked binding of the substrate analog orthovanadate, suggesting that engagement of this carboxy-terminal site influences the active site. Interestingly, analysis by gel filtration and analytical ultracentrifugation revealed that binding of the inhibitor induced a conformational change in PTP1B(1–394) to a more "compact" structure, which was not seen in PTP1B(1–321). In fact, we observed that PTP1B(1–394) displayed resistance to trypsinization in the presence of the inhibitor. Using site-directed mutagenesis, we have identified two binding sites for the inhibitor in PTP1B(1–394) and have now shown that point mutation in two residues, one from each site, was sufficient to abrogate inhibition. The importance of this study is that it suggests a novel mechanism of inhibition that involves the regulatory carboxy-terminal segment of the protein. Furthermore, it supports the presence of an additional binding site(s) for inhibitory small molecules within this noncatalytic portion of PTP1B that would have been missed in the high-throughput screens that have been conducted to date in industry using the 37 kDa form of the enzyme that comprises only the catalytic domain. Currently, we are attempting to initiate high-throughput screens to identify further allosteric inhibitors.

Functional Analysis of JSP1

Previously, we identified JNK stimulatory phosphatase 1 (JSP1) as a dual specificity phosphatase (DSP) that has the capacity to activate the JNK 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 are continuing our characterization of mice that contain a targeted deletion of the *jsp1* gene, particularly with respect to the role of JSP1 in regulating JNK signaling in the neurodegenerative processes that lead to Parkinson's disease and in the role of JSP1 in innate immunity.

In addition to a single DSP catalytic domain, JSP1 contains a potential myristoylation site at its amino terminus (MGNGMXK). Among the members of the PTP family, this is unique to JSP1 and its closest relative DUSP15/JSP2. Consequently, we investigated whether JSP1 was myristoylated and examined the functional consequences of myristoylation. In collaboration with Tom Neubert (Skirball Institute, New York University), we used mass spectrometry to show that wild-type JSP1, but not a JSP1 mutant in which glycine 2 was mutated to alanine (JSP1-G2A), was myristoylated in cells. Abrogation of myristoylation did not impair the intrinsic phosphatase activity of JSP1, but it changed the subcellular localization of the enzyme. Using JSP1 tagged at the carboxyl terminus with 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. Compared to wild type, the ability of nonmyristoylated JSP1 to induce JNK activation and phosphorylation of the transcription factor c-JUN was attenuated. Upon expression of wild-type JSP1, a subpopulation of cells, with highest levels of the phosphatase, was induced to float off the dish and undergo apoptosis. In contrast, cells expressing similar levels of JSP1-G2A remained attached, further highlighting that the myristoylation mutant was functionally compromised. Thus, these studies indicate an important role for myristoylation-dependent localization of JSP1 in the regulation of its signaling function.

Characterization of Novel Cancer Therapeutics

Albert Szent-Gyorgyi, Nobel laureate for the discovery of vitamin C, focused his later years in research on trying to find a cure for cancer and became interested in the properties of flavones in wheat germ. In pursuing this theme, Dr. Mate Hidvegi and his colleagues from Budapest, together with scientists at American Biosciences Inc, have identified a fermented wheat-germ extract termed Avemar®. It has been commercialized in Europe

and the United States as a nutraceutical that displays anticancer and antimetastatic properties. As part of a collaboration involving Dr. Hidvegi and his colleagues, as well as Darryl Pappin and Jim Watson here at CSHL, we have begun studies aimed at identifying and characterizing the active constituents of Avemar®.

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Ming Yang

EMERGING PRINCIPLES OF TUMOR SUPPRESSION

L. Trotman M. Chen A. Naguib
H. Cho D. Nowak
D. Grace C. Pratt
J. Murn M. Zeeman

PTEN is a tumor suppressor that is among the most frequently lost or mutated genes in human cancer. It is unique in directly opposing the enzymatic activity of phosphoinositol-3 kinase (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 a cell-intrinsic human antitumoral defense mechanism. This finding defines a clear role for the p53 gene. It is essential for preventing lethal prostate cancer by mounting the senescence response after *Pten* loss, but it does not initiate tumor growth when lost on its own. Moreover, discovery of the senescence response suggests that tumors should favor retention of some PTEN. Indeed, many human cancers present either with partial loss of the *PTEN* gene or with partial functional impairment. Collectively, these findings have defined *PTEN* as a haploinsufficient tumor suppressor in several tissues and thus emphasize the need for understanding its regulation.

The emergence of patient genome data is now allowing us to probe for genome-wide changes that are consistent with the progression features identified in the mouse. Conversely, we are also probing the mouse tumor genome for spontaneous changes that drive tumorigenesis downstream from their engineered genetic changes. This two-pronged approach is allowing us to move from identification and validation of new cancer genes to connecting the dots by uncovering the principles that underlie *PTEN*-mutant cancer progression.

Nuclear versus Cytoplasmic Akt Activity in Cancer

M. Chen, D. Nowak, C. Pratt, M. Zeeman, 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 promelocytic 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. By studying mice, which are mutant for *Phlpp* or *Phlpp* and *Pten*, we have been able to define *Phlpp* as a tumor suppressor in the prostate. It antagonizes Akt activation at the plasma membrane, and this function is crucial in preventing prostate cancer after partial loss of *Pten*. Intriguingly, combined loss of *Pten* and *Phlpp* triggers the above-mentioned p53 response, and we could show that these tumors need to abolish p53 to form. Thus, we find that the cooperating phosphatases direct Akt activity and localization with entirely differential outcomes with respect to p53 activation. We are currently confirming these findings in human prostate cancer samples.

Nuclear PTEN and Cancer

M. Chen, A. Naguib, M. Zeeman, C. Pratt [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 mech-

anism 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 and in vivo through gene targeting in mouse.

Regulation of PTEN Activity in Prostate Cancer Initiation and Treatment

H. Cho, A. Naguib, J. Murn, M. Zeeman [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 over 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). 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*. Because this finding should form the basis of a therapeutic approach, we set out to test the therapeutic effect of Pten up-regulation in prostate. An important open question behind this approach is that restoration of PTEN might not just antagonize tumor growth, but also revert tumor growth, analogous to the concept of oncogene addiction, even after cells have suffered spontaneous cooperating lesions.

In collaboration with the lab of Dr. Lowe, we have established several mouse models that allow for RNAi-mediated reversible knockdown of *Pten* in prostate. These models now enable us to systematically test if and how established tumors respond to restoration of *Pten*. Thus, they serve as a genetic model for PI3K-inhibitor therapy. We will also test how *Pten* restoration treatment depends on context through genetic alterations, including loss of *Phlpp*, *Pml,* and other cooperating tumor suppressors that we have identified. Through this approach, we have knocked down Pten levels in mouse prostate until tumors have formed with features that are highly similar to those of the traditional knockout models. We are now following up the effects of Pten restoration using magnetic resonance imaging (MRI) methods. Of the 250,000 American men diagnosed with prostate cancer each year, roughly 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

L. Van Aelst B. Boettner, J. Janas, A. Kobayashi, N. Nadif Kasri, R. Packer, C.-L. Wang, Y.-T. Yang

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, their regulators, and effectors in models of neurodevelopmental disorders and cancer. Below are highlighted the main projects that have been carried out during the past year.

The Rho-Linked Mental Retardation Protein Oligophrenin-1 Has a Critical Role at Both the Pre- and Postsynaptic Side of the Hippocampal CA3-CA1 Synapse

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 the 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; however, the pathophysiological role of *OPHN1* has remained poorly understood. 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 is present pre- and postsynaptically. Significantly, we have now obtained data demonstrating a critical role for OPHN1 at both the pre- and postsynaptic sides of the hippocampal CA3-CA1 synapse.

Postsynaptic OPHN1 controls synapse maturation and plasticity by stabilizing AMPA receptors. By temporally and spatially manipulating *OPHN1* gene expression, we have obtained evidence that postsynaptic OPHN1 through its Rho-GAP activity has a key role in the activity-dependent maturation and plasticity of excitatory synapses by controlling their structural and functional stability. Specifically, we found that synaptic activity through *N*-methyl-D-aspartate receptor (NMDAR) activation drives OPHN1 into dendritic spines, where it forms a complex with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and selectively enhances AMPAR-mediated synaptic transmission and spine size by stabilizing synaptic AMPARs. 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 postsynaptic OPHN1 function. Thus, these 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.

Presynaptic OPHN1 controls synaptic vesicle endocytosis via endophilin A1. Neurons transmit information at chemical synapses by releasing neurotransmitters that are stored in synaptic vesicles (SVs) at the presynaptic side. After release, these vesicles need to be efficiently retrieved in order to maintain synaptic transmission. In concurrence, malfunctions in SV recycling have been associated with cognitive disorders. Interestingly, we have found that presynaptic OPHN1 is important for efficient SV retrieval. We demonstrated that reduced/defective OPHN1 signaling impairs SV endocytosis at hippocampal synapses. We further showed

that OPHN1 forms a complex with endophilin A1, a protein implicated in membrane curvature generation during SV endocytosis. Importantly, OPHN1 mutants defective in endophilin A1 binding, or with impaired Rho-GAP activity, failed to substitute for wild-type OPHN1, indicating that OPHN1's interactions with endophilin A1 and Rho GTPases are important for its function in SV endocytosis. These findings also suggest that defects in efficient SV retrieval may contribute to the pathogenesis of *OPHN1*-linked cognitive impairment.

The Rac Activator DOCK7 Has an Important Role in Early Steps of Neuronal Development

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 highlight the contribution of microtubule dynamics to axon development.

More recently, we began to assess DOCK7's role in the development of cortical neurons in vivo. To this end, we implemented the in utero electroporation method that enables highly efficient introduction of vectors coexpressing proteins or short hairpin RNAs (shRNAs) of interest with a fluorescent protein marker, e.g., enhanced green fluorescent protein (EGFP) or tdTomato, into ventricular zone (VZ) cells in embryonic cerebral cortices. We found that either reducing or increasing DOCK7 expression levels in progenitor cells in the embryonic cerebral cortex impairs normal migration of cortical cells, indicating that appropriate levels of DOCK7 are critical for normal migration. Interestingly, we also observed that a significant proportion of Dock7 shRNA-expressing cells remained in the VZ/SVZ several days after electroporation, suggesting that DOCK7 knockdown could as well affect the proliferation and/or differentiation potential of cortical progenitor cells. Indeed, we obtained evidence demonstrating that DOCK7 knockdown in the VZ progenitors increases the number of proliferating S-phase cells and maintains the cells as cycling progenitors, thereby attenuating differentiation of daughter cells into neurons. These findings point to a critical role for DOCK7 in the genesis of cortical neurons. We are currently extending these studies to define the precise mechanistic underpinnings.

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. Rap1 has been assigned critical functions in the morphogenesis of epithelial tissues, and recent studies suggest that dysregulated Rap1 signaling may be intrinsic to malignant processes. However, how Rap1 controls epithelial morphogenesis in vivo has remained largely elusive. We have identified a signaling module that contains PDZ-GEF, a Rap1-specific exchange factor, Rap1 itself, and the adaptor protein AF-6. By investigating developmental events in *Drosophila* as a genetically tractable model system, we demonstrated that PDZ-GEF, Rap1, and Canoe, the *Drosophila* AF-6 ortholog, function in a linear pathway that governs epithelial migration and gastrulation events in the embryo, as well as cell shape in expanding imaginal discs, the precursors to diverse adult structures. We found that the activity of this pathway in part impinges on Myosin II as a motor protein that modulates cell shape in epithelial morphogenetic processes.

More recently, we have uncovered an additional role for AF-6/Canoe as a Rap1 effector in mitotically active epithelia, such as imaginal discs. Our experiments show that AF-6/Canoe is key to a mechanism that reconstitutes E-cadherin-based adherens junctions at the apical membrane between sister cells late in cytokinesis. A failure in this process causes cells to aberrantly disperse and missort in their respective tissue domains. By conducting further genetic analysis, we found that AF-6/Canoe in this context acts on its interacting protein Echinoid,

a *Drosophila* homolog of the Nectin family of IgG adhesion proteins. Echinoid expands the apical adherens junctional belt to its normal circumferential size downstream from AF-6/Canoe. Surprisingly, our functional dissection also suggests that an independent function of Echinoid acts in parallel to AF-6/Canoe in the general maintenance of adherens junctions. Our observations thus provide insights into a novel molecular framework that perpetuates adherens junctional continuity in mitotically active epithelia.

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, 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.

Whereas our data showed that Dok-1 opposes $p210^{Bcr\text{-}Abl}$-driven leukemogenesis, the $p210^{Bcr\text{-}Abl}$-induced disease does eventually progress with time. That this progression takes place implies that Dok-1 function eventually becomes incapacitated. 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 cells lines and that the reduction in the levels of Dok-1 protein requires tyrosine kinase activity of these oncogenes. Our preliminary data indicate that oncogene tyrosine-kinase-induced Dok-1 down-regulation involves proteasome-mediated proteolysis. We are currently corroborating these data and investigating the precise underlying molecular mechanism(s). 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

CSHL neuroscientists seek to understand the means by which the developing brain gives rise to complex neural networks underlying memory, behavior, and cognition. In addition to its investigations into the functioning of the healthy brain, the Laboratory is engaged in the discovery of genes implicated in mental illnesses and the determination of how mutations in these genes influence brain function in animals. This, in turn, is envisioned as the basis for a profound understanding of brain abnormalities associated with diseases such as Alzheimer's, Parkinson's, autism, schizophrenia, and depression, new knowledge that will form the basis for the next generation of treatments.

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, many of which are likely to 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. Both types of traces, the team discovered, depend on the activity of a gene called *rutabaga*. Dubnau's team also explores how groups of genes interact to form memories, an approach designed to shed light on complex gene networks whose dysfunction 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 they are using these models to determine the targets of antidepressant therapies, identify signaling pathways that control generation of new neurons, and 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 explains why the number of new neurons decreases with advancing age. Enikolopov's group is also 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 humans and animals. The lab is now using these discoveries 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: NMDA receptors and calcium homeostasis modulators (CALHM). Both are involved in 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 neurotransmitter ligands and allosteric modulators in different subtypes of NMDA receptors in order to provide a blueprint for future drug design. This year, they published the crystal structure of the extracellular region of the NR2B subunit of the receptor, which includes an amino-terminal domain whose uniqueness makes it a potentially interesting drug target. The team is also working to reveal the molecular architecture of CALHM in order to determine the mechanism by which this calcium-specific ion channel opens. Such structural information promises to be useful in the design of novel drugs for treatment of Alzheimer's, which has been linked to a mutation of the gene coding for CALHM.

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 partic-

ularly 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 Rhett's syndrome, one of the autism spectrum disorders.

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, optical-genetic manipulations, and quantitative analysis. They are currently pursuing two broad directions. First, they seek to elucidate the principles of decision-making, and in particular how the brain computes and uses confidence estimates about decisions. Second, they want to understand how specific cell types and neural pathways participate in the neural circuit dynamics underlying decision processes. Toward these goals, they conduct electrophysiological recordings of large populations of neurons in behaving mice and rats. In addition, they are adapting optical and genetic tools for use in behaving animals in order to manipulate specific neural cell types and pathways with the long-term goal of causally linking the activity of specific neural types and pathways to behavioral decisions.

Alexei Koulakov and colleagues are trying to figure out 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 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, RNA-interference-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. A new project focusing initially on the gene *ErbB4* seeks to determine the genetic causes of NMDA receptor hypofunction, a pathology believed to contribute to the etiology of schizophrenia.

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 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 year, Mitra received funding from the National Institutes of Health for a multiyear project that will culminate in a first-draft mesoscopic-scale circuit map of the entire mouse brain. This is contemplated as a first step to mapping vertebrate brain architecture across species. Separately, he and colleagues published results of neurobiology research demonstrating the de novo evolution of song culture in zebra finches, starting from an isolate population.

Pavel Osten's lab works on identification and analysis of brain regions, neural circuits and connectivity pathways that are disrupted in genetic mouse models of autism and schizophrenia. Osten hypothesizes that (1) systematic comparison of many genetic mouse models will allow determination of overlaps in pathology—neural circuit endophenotypes—responsible for the manifestation of neuropsychiatric disorders and (2) neuron-circuit-based classification of autism and schizophrenia will provide key circuit targets for detailed mechanistic studies and therapeutic development. Osten and colleagues are developing the first systematic approach to the study of neural circuits in mouse models of psychiatric diseases, based on a pipeline of anatomical and functional methods for analysis of mouse brain circuits. An important part of this pipeline is high-throughput two-photon microscopy for whole-mouse brain imaging. Other methods include virus-vector-based anatomical tracing, transgenic and knock-in "indicator" mouse lines for monitoring expression of activity-regulated genes, and both in vitro and in vivo two-photon imaging and electrophysiology.

Jonathan Sebat's laboratory studies 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 a rare mutation associated with dramatically increased schizophrenia risk. The mutation is a gene copy-number variation in a chromosomal region, 16p11.2, already linked to autism. Previously, they had demonstrated a significantly increased rate of rare structural mutations in the genomes of people with schizophrenia as compared with healthy controls. They found, moreover, that the mutations were powerful and that 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 includes many different rare mutations, each one present in comparatively few individuals, or even a single one. In prior work, Sebat, in collaboration with Michael Wigler, discovered that spontaneous mutations—genetic errors in children that do not occur in either parent—are far more common in autism than previously thought.

Stephen Shea's lab studies the neural circuitry underlying social communication and decisions. He uses natural social communication behavior in mice as a model to understand circuits and processes that are evolutionarily conserved and therefore shared broadly across species, likely contributing to disorders such as autism. This past year, Shea and colleagues examined how emotion and arousal enable mice, via their olfactory systems, to store memories of other individuals and of related social signals. The team succeeded in exploiting the tight neurobiological linkage between emotion and arousal, on the one hand, and memory, on the other hand, to effectively create memories in anesthetized mice. They were then able to study properties of the created memories in great detail, linking mechanisms of neuromodulation with those of neural plasticity and behavior. The lab's immediate objective is to make targeted recordings from different neurons in the circuit and image the activity of large groups of neurons as memories are being formed, to see how they interact to produce the neuronal firing-rate changes that presumably store memories. Ultimately, the Shea lab aims to include recordings from awake, behaving mice and other techniques to understand this process all the way from the level of a live social encounter to the movement of neurotransmitter receptors at the synapse.

What is a memory? When we learn an association, information from two different sensory streams must somehow be bound together in the brain. For example, we may learn the smell of an unripe banana predicts its starchy taste. What changes in neural activity underlie this learning—

what synapses change strength, what ion channel properties change? How do these changes alter cellular and network-level responses so our perception of the odor is linked to the aversive reaction to the starchy taste? These are the questions that drive research in Glenn Turner's laboratory. His team addresses these questions by monitoring neural activity using a combination of electrophysiological methods to examine individual neurons with very high resolution and functional imaging to track activity of hundreds of neurons simultaneously. To test how the network functions, they use molecular genetic techniques to manipulate activity of specific neurons in learning and memory centers of the fly brain. They also examine the effects of learning-related genes on neuronal response properties. In this way, they directly test their predictions about neural coding, both at the level of spike trains and at the level of animals' perception.

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 reacted strongly when exposed to a specific sound, the lab challenged the standard model of sound representation. Zador's team has 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 three 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. Recently, they succeeded in linking specific genetic defects in Noonan's with long-term memory deficiencies that are among the symptoms of the illness. In fly models, they discovered the molecular underpinnings of an observed "spacing effect"—the fact that learning tends to be preserved longer in memory when learning sessions are spaced out between rest intervals. They demonstrated that a protein, SHP-2 phosphatase, controls the spacing effect by determining how long resting intervals between learning sessions need to last so that long-lasting memories can form. Zhong's team has succeeded in reversing memory deficits in mutant flies, work that suggests longer resting intervals for Noonan's patients might reverse their memory deficits.

GENETICS OF MEMORY IN *DROSOPHILA*

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

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-term 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, L. Prazak

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]), including 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, including *staufen* and *oskar*, which are known components of a 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., *Alcohol Clin Exp Res 32*, 895 [2008]; Chen et al., *PLoS Comput Biol 4*, e40 [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 at CSHL) to predict *pumilio* targets among mRNAs whose products have synaptic localization (Chen et al., *PLoS Comput Biol 4*, 340 [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 endogenous levels of *dlg1* (Chen et al., *PLoS Comput Biol 4*, 340 [2008]) and another neuronal target, 14-3-3, can be regulated by *pumilio* in a neuronal context, the adult mushroom bodies (MB), which is an anatomical site of memory storage (Fig. 1 and

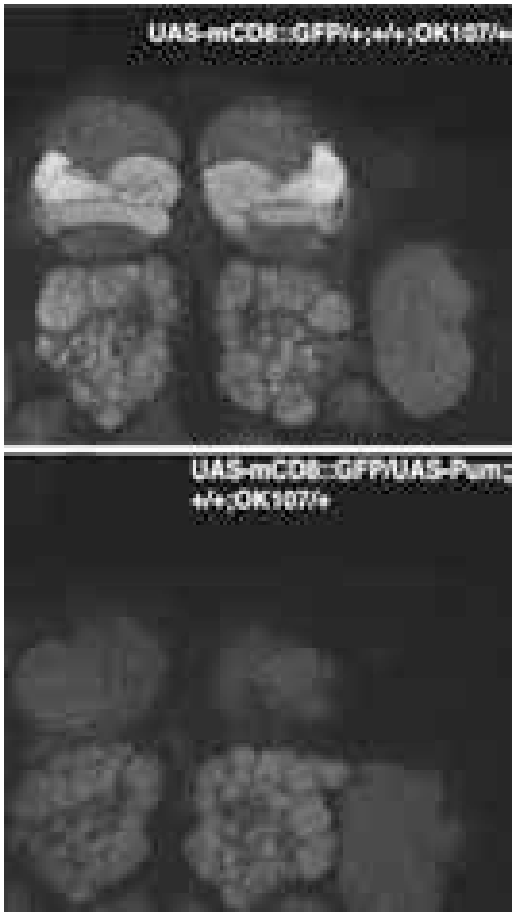

Figure 1. Dlg is repressed by overexpression of Pum in MB Kenyon cells. An MB-expressing Gal4 line (OK107) was used to drive expression of both UAS-mCD8::GFP (Green) and UAS-Pum transgenes. Shown are optical sections of the MB lobes. In wild-type (*top*), Dlg expression is detected both in MB lobes and 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.

data not shown). 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.

Neural Circuitry of Memory

A. Blum, M. Cressy, H. Qin, 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 mushroom body (MB). The Ca^{2+}-responsive adenylyl cyclase *rutabaga* is believed to be a coincidence detector in γ neurons, 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 et al. 2009). 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-term 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 et al. 2009; Blum and Dubnau 2010). Our current efforts focus on elucidation of the signaling differences relevant to learning that lie downstream from the DA1 dopamine receptor within these two neuronal cell types.

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 the genetic background can have profound impacts on phenotype. In addition to its obvious importance to our understanding of genotype-to-phenotype relationships, the "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) protein (CBP) located in

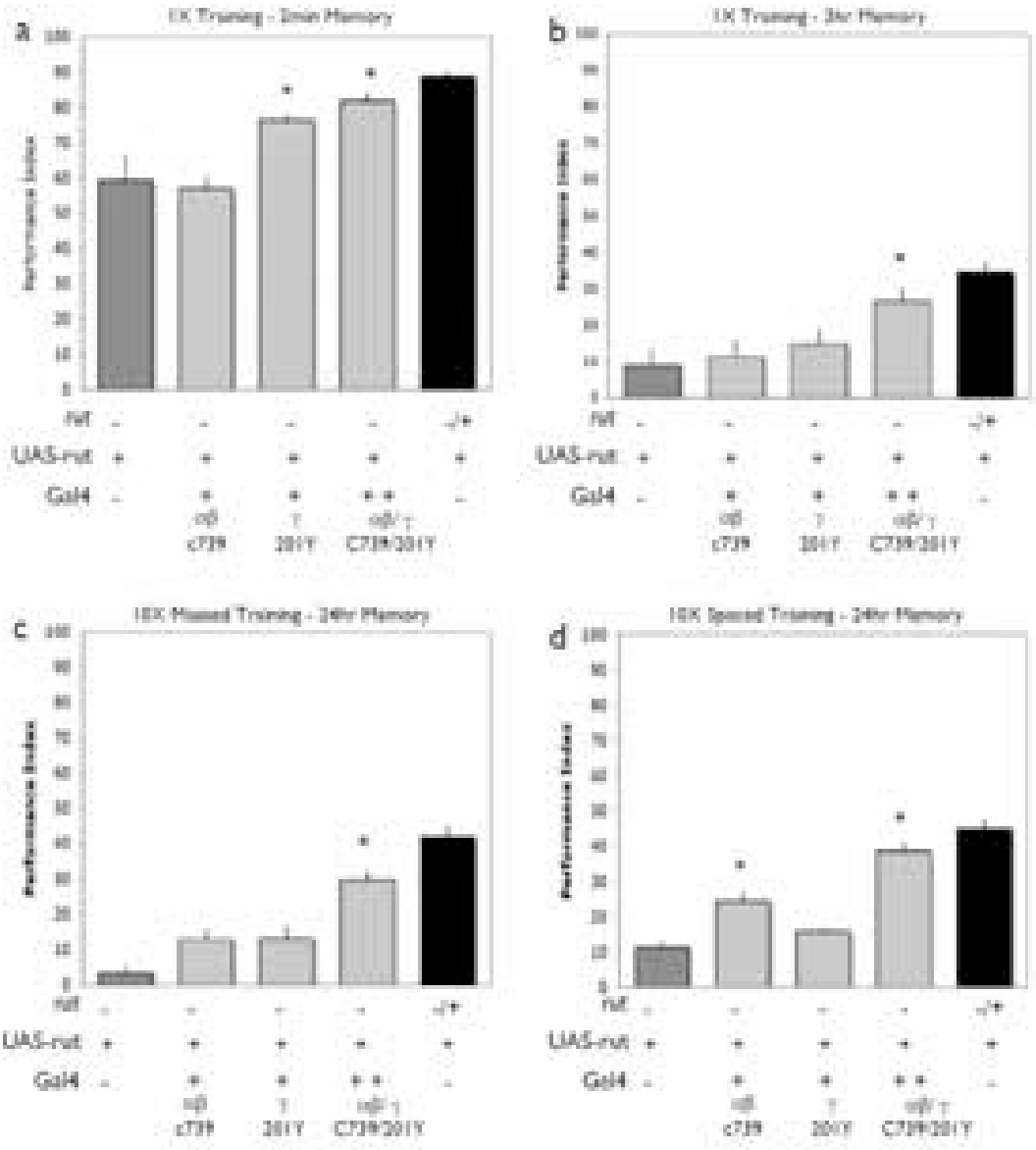

Figure 2. *rutabaga* expression in γ neurons supports short-term memory but not long-term memory. In contrast, *rutabaga* expression in α/β neurons supports long-term but not short term memory. Memory retention was tested 2 min (*a*) and 3 h (*b*) after a single training session as well as 24 h after 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 driver 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.

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-wise gene interactions via modifier screens. Although this strategy has been tremendously successful at revealing the 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 also is 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 first were 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.

We used selective breeding for improved memory (rutabaga suppression) on three replicate populations for 41 generations (Fig. 3). Control populations were allowed to drift in parallel. A robust response to selection is seen beginning at about generation 13. We then used a high-throughput genotyping to determine allele frequencies and coinheritance patterns for all 23 loci for 288 animals per population across the replicate populations (Fig. 3). Multivariate analyses of these high-dimensional data examined *which* of the 23 loci were responsible for the phenotypic effect. Samples with more than 30% failed reactions were discarded, and values for the remaining missing data were assigned by drawing from the empirical genotype distributions of the valid calls. This procedure (imputation) was repeated 100 times to ensure an accurate depiction of the allele frequencies. The allele frequencies showed little variance over the 100 imputations (data not shown). A singular value decomposition (SVD) was then performed on each imputed matrix. The SVD is typically used for dimensional reduction and is also the algorithm used in principal component analysis (PCA). The SVD, however, is a matrix factorization technique that need not be interpreted in statistical terms (as in PCA). An SVD provides orthonormal bases for both the column space and the row space of the original data matrix. In our case, we obtain a "sample space" effectively describing the genotypes of typical flies and an "allele space," effectively describing the relevant allelic contributions.

Having obtained these descriptive spaces, linear discriminant analysis (LDA) was used to discriminate between selected and unselected groups across generations in the reduced sample space. LDA defines the direction of separation between selected and unselected populations (Fig. 3, middle panel), and when projected into "allele space," this vector gave a list of weights describing the contribution of each allele to the phenotypic effect (Fig. 3, bottom panel). Positive weights suggest alleles beneficial to the memory task, and negative weights suggest alleles detrimental to the memory task. Neither the SVD nor LDA showed significant differences over the 100 imputations to fill in missing data (due to lack of genotype calls), lending support to the robustness of the method.

These data identify eight alleles that are predicted to underlie the selection response. We used standard Mendelian genetics to generate flies that were homozygous for the *rutabaga*^{1} allele and heterozygous for one of the transposon mutations identified in the SVD (Fig. 3). This experiment has been completed for each of the eight elevated alleles and for the one allele that is most dramatically elevated in the UNSELECTED direction (E1847).

In each case, we compared memory performance to that of the *rutabaga* mutant to test for suppression (improvement in learning). We also tested the effects in animals that were heterozygous for the recessive *rutabaga*^{1} mutant. We find that the learning performance of null *rutabaga* animals is significantly improved if these animals also are heterozygous for any of the top three alleles (D0077, E3272, or E3945) identified from the SVD (Fig. 3). In contrast, memory is not significantly improved or decreased with the other five of the top eight alleles (Fig. 3 and data not shown) or with E1847 (Fig. 3), which was the allele significantly opposing the separation. These data serve as an independent confirmation that several of the identified alleles are causally related to the memory performance improvement in the selected lines and that the SVD analyses are predictive of the behavioral outcomes. It is gratifying that the three highest-impact alleles in the multivariate analysis are each *individually* capable of partially suppressing the *rutabaga* learning defect. Current efforts on this project focus on testing the following four hypotheses:

- **Hypothesis 1:** Multiple genetic solutions underlie the response to selection.
- **Hypothesis 2:** Multigene interactions contribute to the selection response.
- **Hypothesis 3:** Genetically different "solutions" to selection share mechanistic similarities.
- **Hypothesis 4:** The separation between selected/unselected groups found in the analysis is due to selection in the memory task and not to a founder effect, neutral drift, or natural selection.

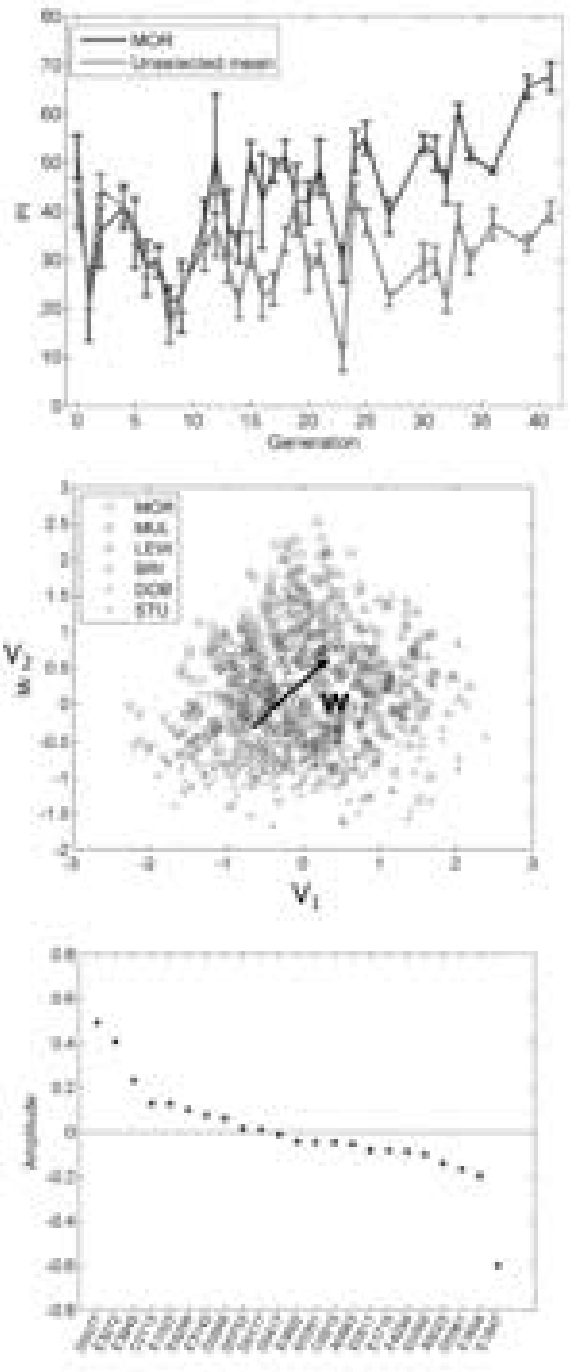

Figure 3. Experimental evolution of *rutabaga* suppression. (*Top panel*, MOR) Performance for one or the three selected lines (Mor) over the course of 41 generations; mean data for three selected lines are shown for comparison. Similar responses are seen in each of the three unselected populations (not shown). Importantly, no response to selection was observed in a control population with no variation at the 23 loci (not shown). (*Bottom two panels*) Multivariate analyses of the genotype data. The first three components in the SVD account for ~40% of the variance. The SVD transfers each genotype into a three-dimensional meta-genotype space in which the directions are no longer allele dosages, but instead, some representation of genotypes more related to genotype interactions. When each animal is plotted in the meta-genotype space (*middle panel*), there is a definite separation between the groups (similar results at generation 11; not shown). LDA was used to give the direction of separation (w). Because the SVD also gives a corresponding meta-allele space, we can project the allele profile vectors onto w, giving the amplitude of contribution of each allele to w. Allele contributions (*bottom panel*) are sorted so that largest effects are to the left. Alleles above the zero line are predicted to cause the separation between selected and unselected groups.

Extinction Learning: A Model for Posttraumatic Stress Disorder

H. Qin, J. Dubnau

Repeated representation of the conditioned stimulus (CS) without the reinforcement of the unconditioned stimulus (US) results in the diminishment of conditioned responding. This process is called extinction (of

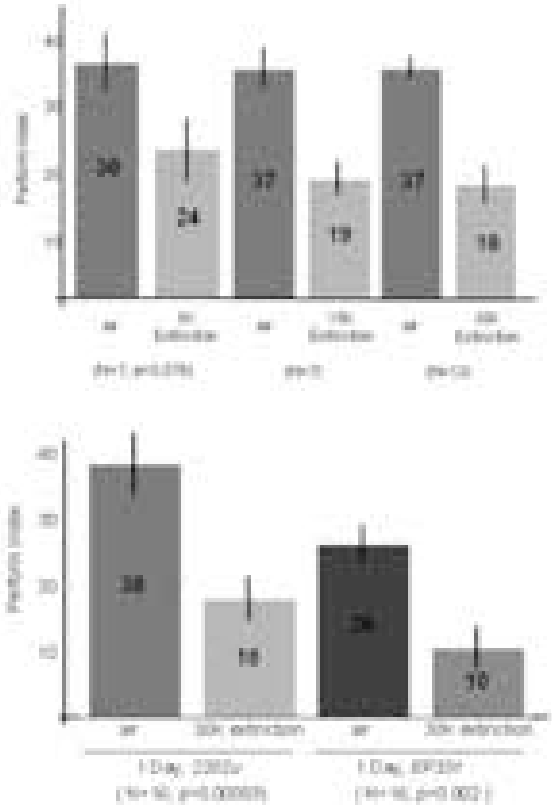

Figure 4. Extinction of "fear" conditioning in *Drosophila*. Extinction paradigm (*top panel*) involves three stages. First, animals are trained in a Pavlovian olfactory avoidance assay using 10 sessions of spaced training. This leads to a robust and stable long-term "fear" memory. Second, 24 h after training, animals are subjected to varying numbers of extinction learning sessions. These consist of repeated exposure to the odors in the absence of the electric shock reinforcement. Finally, the animals are tested for avoidance of the conditioned odor. Using 5, 15, or 30 sessions of extinction, we observe a robust extinction of the fear avoidance. 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) and NMDAR1 (*bottom panel*). NMDAR1 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).

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. This type of learning is important biologically, but it also has 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 observe a robust extinction learning in mutations that disrupt either cAMP signaling or *N*-methyl-D-aspartate (NMDA) receptor function. We also observe extinction in mutations that disrupt either short-term memory or long-lived memory. In contrast, genetic disruption of the development of a single neural cell type appears to block extinction (Qin and Dubnau 2009). These results establish an entry point to dissect the neural circuits that serve the fear conditioning versus extinction.

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In Press

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STEM CELLS, SIGNAL TRANSDUCTION, AND BRAIN FUNCTION

G. Enikolopov T. Michurina N. Peunova
J.-H. Park H. Louis dit Picard

We study stem cells, their contribution to tissue plasticity, and the signals that control their fate. Our main focus is on stem cells of the brain and the neuroendocrine tissues. We use animal models to study the fate of stem cells and to learn how physiological stimuli, aging, disease, and therapies affect stem cells and the tissue where these cells reside. Our particular interest is in mechanisms that control stem cells' quiescence and entry into division cycle. 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). We are also working to translate the knowledge we generate with animal models to human studies.

Neural Stem Cells and Brain Disorders

New neurons are continuously generated in restricted areas of the brain; one is the hippocampus, a region crucial for cognitive function. New neurons are born from neural stem cells as a result of a complex cascade of events including 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. Production of new neurons from stem cells is a highly dynamic process and can be modified by 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 to determine the targets of important proneurogenic or antineurogenic stimuli. Our main approach is to generate transgenic reporter animal lines, to combine them with other genetically modified lines, to use these complex compound lines to dissect the neurogenesis cascade into distinct stages, and then to identify the steps that are specifically affected by a particular stimulus. We used this approach, aided by computational modeling (in collaboration with Dr. Alex Koulakov, CSHL), to produce a new scheme of the stem cell life cycle. We are using this scheme to address the basic mechanisms that control the quiescence, self-renewal, differentiation, and death of stem cells in the adult and aging brain. Furthermore, 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 have already used this scheme to identify the targets of various antidepressant drugs and treatments, exercise, radiation, chemotherapeutic agents, trauma, Parkinson's disease, and aging. In particular, we found that diverse antidepressant therapies such as fluoxetine, exercise, and deep brain stimulation of distant brain regions converge on the same downstream step of the cascade, without affecting the stem cells; in contrast, electroconvulsive shock, another antidepressant therapy, activates division of stem cells.

We have also found that trauma and disease can activate stem cells of the hippocampus, even when distant regions of the brain are damaged. In particular, we found that moderate traumatic brain injury (with cortical impact) promotes division of stem cells in the adult hippocampus. Furthermore, in the model of Parkinson's disease, we found that ablation of dopaminergic neurons of the substantia nigra (which project to the hippocampus) can also activate hippocampal stem cells. Together, these results suggest that even distant events such as a trauma of the cortex or ablation of neurons in the midbrain can activate neural stem cells of the hippocampus; such activation may reflect the induction of innate repair and plasticity mechanisms by the injured brain.

Our results also suggest an explanation of the age-related decline in hippocampal neurogenesis. Age is the major factor that affects neurogenesis, and, given the potential significance of new neurons for cognitive function, it has been hypothesized that reduced neurogenesis may contribute to age-related cognitive impairment. Our results indicate that a decrease in the number of new neurons in the hippocampus is a consequence of a decrease in the number of neural stem cells. Moreover, the decrease in stem cells is a division-coupled process and is a direct consequence of their functioning to produce new neurons. We now 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.

The changes that neural stem cells undergo during aging or disease suggest that being able to detect variations in the level of neurogenesis in the live brain may be important for diagnostic purposes. In collaboration with Dr. Helene Benveniste (Stony Brook University and Brookhaven National Laboratory), we are working to characterize changes in magnetic resonance spectra of the live animal and human brain that would be indicative of the changes in neurogenesis. Moreover, this approach is now being investigated in a number of preclinical and clinical trials, and it may become useful for following neurological and psychiatric disorders and cancer.

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. Toward the goal of identifying, isolating, and tracking adult stem cells, we generated a series of compound reporter transgenic mouse lines where stem and progenitor cells and their progeny in a number of tissues are marked by fluorescent proteins. Interestingly, the same reporter lines that highlight stem cells of the developing and adult nervous system also help to highlight stem and progenitor cells in a range of other organs and tissues, including nonneuronal tissues. The list now includes stem cells of such dissimilar structures as brain and spinal cord, hair follicles, liver, pancreas, skeletal muscle, testis, ciliary margin of the eye, anterior pituitary, and mesenchymal stem cells. This observation points to a close (and yet unexplained) relation 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.

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 stromal cells and produces NO, which acts as a paracrine regulator of hematopoietic stem cells.

We continue to discover the diversity of biological functions of NO. Our current work focuses on the role of NO as a regulator of morphogenesis during early development. We found 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 overlapping but separate signaling pathways. Concurrent control by NO helps ensure that the crucial processes of cell proliferation and morphogenetic movements are coordinated during early development.

We have now discovered yet another role of NO and nNOS during early development: its function as a regulator of planar polarity of cilia. We demonstrated planar polarity of the distribution of cilia in the *Xenopus* larvae and found that NO and XNOS1 (an ortholog of nNOS) are important mediators of this polarity. We also found a new mode of establishing planar polarity by function-dependent stabilization of cilia growth. Moreover, we demonstrated that the role of NO in mediating planar polarity of cilia extends to mammalian tissues, highlighting its role in human health and disease.

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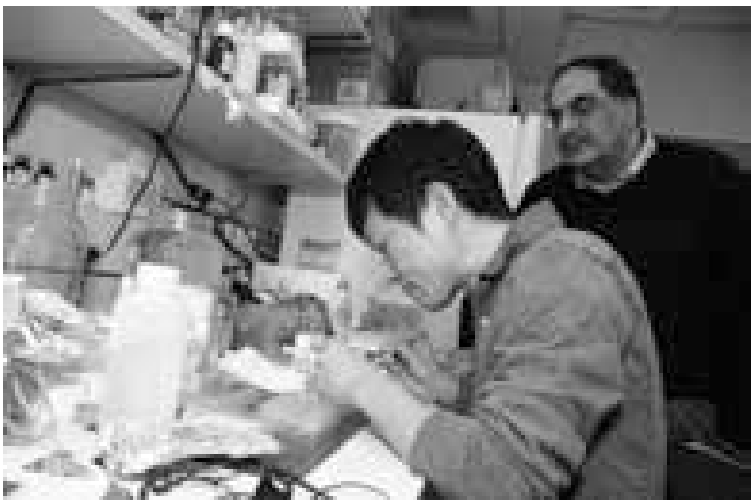

J.-H. Park and G. Enikolopov

MOLECULAR ANALYSIS OF NEURONAL RECEPTORS AND ION CHANNELS

H. Furukawa E. Karakas
N. Simorowski
M. Yuchi

The research in my group is aimed at understanding the molecular basis for the function of cell surface proteins that trigger cellular signal transductions involved in neurotransmission in the mammalian brain. Our goal is to help design compounds with therapeutic potentials in neurological diseases and disorders including schizophrenia, depression, stroke, and Alzheimer's disease. Toward this end, we are conducting structural and functional studies on neurotransmitter receptor ion channels and intramembrane enzymes, which facilitate and modulate 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 three classes of cell surface proteins, *N*-methyl-D-aspartate receptor (NMDAR), calcium homeostasis modulator (CALHM), and γ-secretase, dysfunctions of which are highly implicated in the pathogenesis of Alzheimer's disease.

Structure and Function of NMDARs

NMDARs belong to the family of iGluRs, which mediate the majority of excitatory synaptic transmission in the mammalian brain. Dysfunctional NMDARs are implicated in neurological disorders and diseases including seizure, stroke, schizophrenia, Parkinson's disease, and Alzheimer's disease. NMDARs are multimeric ligand-gated ion channels composed of the NR1 and NR2 subunits, which bind glycine and L-glutamate, respectively. Unlike non-NMDARs that can form functional ion channels as homotetramers, the NMDARs can only function as the NR1-NR2 heteromultimers, and opening of the ion channels requires binding of both glycine and glutamate to NR1 and NR2, respectively. The four distinct NR2 subunits control ion channel properties and show different spatial and temporal expression patterns; thus, there has been enthusiasm to create subtype-specific compounds that can target specific neuronal circuits.

The NMDAR subunits are modular proteins composed of the amino-terminal domain (ATD), ligand-binding domain (S1S2), transmembrane domain (TM), and carboxy-terminal domain (CTD) (Fig. 1). The domain of focus in this study is the ATD at the extracellular region, which mediates allosteric regulation of the NMDAR ion channel activities. From the pharmaceutical perspective, most of the compounds that target the NMDAR S1S2 ligand-binding cores (Fig. 1) cause severe side effects due to nonspecific binding to different NMDAR subtypes. This stems from high sequence

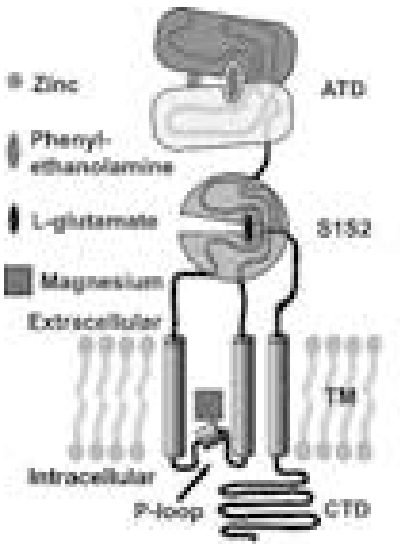

Figure 1. Domain organization of NMDAR subunit. The amino-terminal domain (ATD) at the extracellular region is followed by S1, transmembrane region (TM) 1 and 2, P-loop, the S2 segment, TM 3, and carboxy-terminal domain (CTD). The S1 and S2 peptides form a ligand-binding core that binds agonists and antagonists. ATD in NMDARs bind allosteric modulators (zinc and phenylethanolamines). TM forms the ion channel in the context of the heteromultimeric assembly of NR1 and NR2 subunits, which is blocked by magnesium at resting membrane potential. CTD interacts with intracellular proteins such as PSD95 and CAMKII to mediate cellular signal transduction important for synaptic plasticity.

identity and therefore, high structural similarity within the S1S2 ligand-binding domains of the NR2 subunits (namely, NR2A–NR2D). In contrast, sequence identity is moderate (30%–40%) in ATD, thus making this domain an attractive drug target. Indeed, a group of compounds called phenylethanolamine and zinc specifically bind NR2B ATD and allosterically inhibit the activity of the NR2B containing NMDARs. To date, the molecular mechanism for the ATD-mediated regulation is not well understood, and structural information for the NMDAR ATD has not been available. Our group has recently solved the structure of NR2B ATD and answered questions involving the molecular mechanism of ATD-mediated allosteric inhibition.

We have completed X-ray crystallographic studies on NR2B ATD in two states: zinc-bound states and the apo state. This X-ray crystallographic study has revealed that the NR2B ATD has a clamshell-like overall architecture composed of two lobes, which we call the R1 and R2 domains (Fig. 2). Using the anomalous dispersion method in X-ray crystallography, we have been able to unambiguously identify a binding site for a zinc ion deep in the clamshell cleft. The clamshell structure is found in the closed conformation where the zinc ion at the cleft appears to induce the global domain closure. Indeed, mutation of residues involved in zinc coordination as well as the R1-R2 interdomain interaction hampered the extent of zinc-mediated allosteric inhibition on the intact NR1/NR2B NMDARs as assessed by two-electrode voltage-clamp (TEVC) experiments. We have also spotted a region in the clamshell cleft that is clustered with hydrophobic residues. To understand the potential functional roles of the hydrophobic residues, we have conducted systematic structure-based mutagenesis on those residues in combination with TEVC (Fig. 3). We have found that mutations of hydrophobic residues have a significant effect on potency and efficacy of phenylethanolamine-mediated allosteric inhibition but have little or no effect on zinc-mediated allosteric inhibition. These results have suggested that zinc and phenylethanolamine have distinct binding sites at the clamshell cleft even though they both mediate allosteric inhibition. To verify this functional hypothesis, we are currently attempting to solve the NR2B ATD structure in complex with phenylethanolamine compounds including ifenprodil. We are continuing with our effort to understand the mechanism by which bindings of zinc and/or phenylethanolamines to NMDARs inhibit ion channel activities by further conducting X-ray crystallographic studies on the NR1/NR2B ATD complex.

Structure and Function of Calcium Homeostasis Modulator

Calcium homeostasis in neuronal synapses is maintained by the cell surface and endoplasmic reticulum

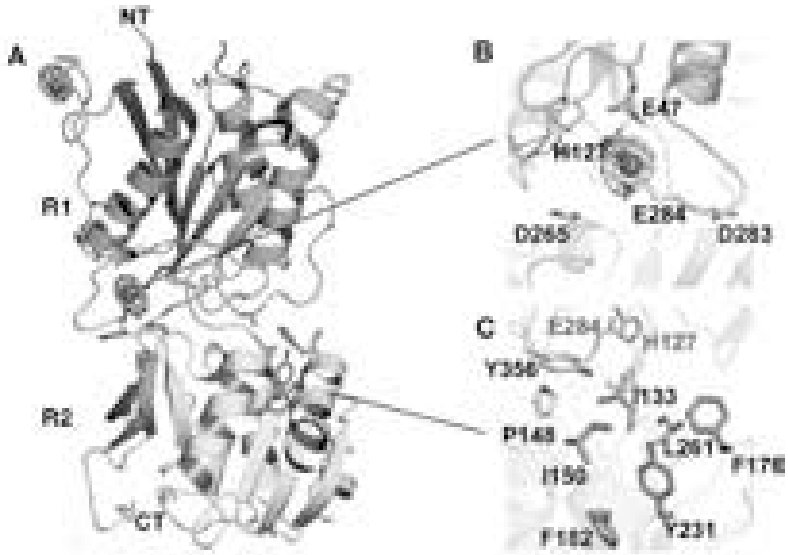

Figure 2. Crystal structure of NR2B ATD in complex with zinc. (*A*) Overall architecture of NR2B ATD composed of two lobes, R1 (*top*) and R2 (*bottom*) domains. Zinc sphere in mesh area is coordinated by hydrophilic residues at the cleft sticks at top. A patch of hydrophobic residues is observed on the opposite side of the clamshell cleft sticks at bottom. (*B,C*). Enlargement of the zinc-binding site (*B*) and hydrophobic site responsible for binding of phenyethanolamine compounds (*C*).

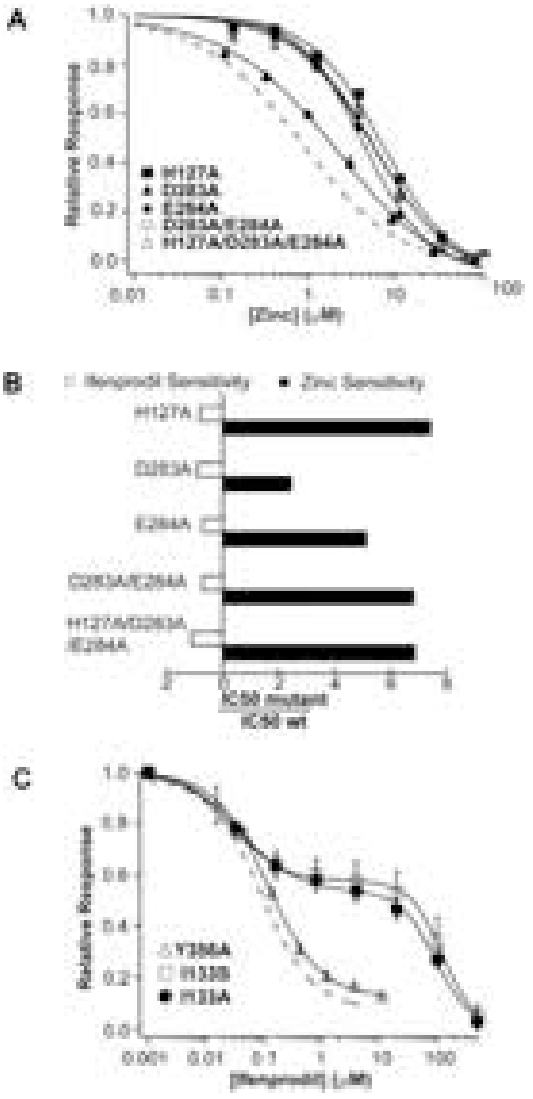

Figure 3. Validation of zinc- and ifenprodil-binding sites. (*A*) Dose response of zinc-mediated inhibition on the wild-type and mutant NR1/NR2B NMDAR ion channels as assessed by TEVC held at –20 mV membrane potential. (*B*) Residues affecting zinc inhibition do not have any effect on phenylethanolamine sensitivity. Values in the bar graphs are IC_{50} of zinc and ifenprodil inhibitions. (*C*) Mutagenesis of I133 located in the middle of the hydrophobic site showing a large shift in IC_{50} value upon mutation.

(ER) membrane proteins including NMDARs, voltage-gated calcium channels, IP3 receptors, and the recently identified CALHMs. 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). Currently, there is little or no knowledge on the functional properties of CALHM. A moderate degree of sequence similarity to NMDARs at the transmembrane region suggests that the CALHM ion channels are calcium-permeating ion channels. However, whether or not CALHM ion channels are regulated by external ligands is not known. Thus, by using biochemical, electrophysiological, and structural biological approaches, we are aiming to dissect the function of CALHM at a molecular level. We have, so far, been able to express and purify CALHM proteins with a size homogeneity suitable for crystallographic studies. We plan to conduct X-ray crystallographic studies to obtain atomic structures and electrophysiological experiments to characterize the ion channel property of CALHM.

Structure and Function of γ-Secretase

γ-Secretase is a multimeric protein complex of four transmembrane proteins including presenilin, nicastrin, APH-1, and PEN-2. Together, the complex mediates the paradigm of a molecular event called regulated intramembrane proteolysis, which is a type of proteolysis that occurs within the membrane. Intramembrane proteases are involved in numerous cellular processes including cholesterol homeostasis, development, immuno-surveillance, and Alzheimer's disease pathogenesis. γ-Secretase cleaves various single-transmembrane substrates including amyloid precursor protein and Notch ligand. A mechanistic understanding of the activities in γ-secretase and intramembrane proteases in general is restricted due to limited numbers of available crystal structures. Structural information about γ-secretase is currently limited to low-resolution images obtained by single-particle analysis using cryoelectron microscopy. Our group is interested in revealing atomic structures of γ-secretase to gain insights into intramembrane proteolysis, substrate specificity, inhibition, and functional modulation. Currently, we are working toward understanding the mechanism of substrate recognition mediated by one of the γ-secretase components, nicastrin.

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CONSTRUCTION AND FUNCTION OF GABA INHIBITORY CIRCUITS IN NEOCORTEX

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

The neocortex is the crowning achievement of evolution and subserves our uniquely human abilities. Although mental functions emerge from interactions between multiple distinct neocortical areas, cortical networks consist of stereotyped modular circuits, subtly specialized across regions and mammalian species. A key obstacle to understanding how cortical circuits perform complex computations is the stunning diversity of neuron types and a lack of knowledge about the basic biology of their construction. Although a minority, GABAergic interneurons are crucial in establishing the functional balance, flexibility, and computational architecture of cortical circuits. Understanding the origin, organization, and function 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 has been a major challenge to studying cortical GABAergic interneurons using conventional anatomical and physiological techniques. The genetic approach is ideal for dissecting 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 (gene targeting and Cre/loxP recombination-regulated gene expression), we have generated ~20 knockin "driver lines" expressing Cre or inducible CreER to establish "genetic access" to all major classes GABAergic neurons. Characterization of GABA Cre drivers indicates that our genetic strategy captured most of the anatomically and physiological defined cell classes. In addition, an intersectional strategy combining Cre and Flp lines further increased specificity, suggesting that highly distinct cell types can be captured by overlapping two driver systems. These genetic tools allow reliable and systematic visualization and functional manipulation of defined GABAergic cell types, and they also enable cell-type-based genomic analysis of their molecular profiles. A number of driver lines allow us to visualize the "life history" of subsets of GABA neurons (e.g., dendrite-targeting somatostatin interneuron), from their birth in ganglionic eminence to their action in mature cortical circuits. We are examining whether and how cell lineage and developmental history influence the assembly of GABA neurons into cortical circuits. These driver mice will significantly accelerate progress in studying all aspects of the GABAergic system.

Activity-dependent Maturation and Plasticity of Inhibitory Synapses and Circuits

GABA-mediated synaptic inhibition is crucial in neural circuit operations. The development of inhibitory synapses and innervation patterns in the neocortex is a prolonged process, extending well into the postnatal period, and is regulated by neural activity and experience. Such activity-dependent inhibitory synapse development is a major component of the functional maturation of inhibitory circuits, which organize neural ensemble and network dynamics, but the underlying mechanisms are poorly understood. We previously provided evidence for the hypothesis that GABA signaling acts beyond synaptic transmission and regulates inhibitory synapse development; in other words, similar to glutamate signaling at developing excitatory synapses, GABA may coordinate presynaptic and postsynaptic maturation at inhibitory synapses.

In the past year, we have made significant progress in understanding how presynaptic $GABA_B$ receptors and neurexin (NRX)–neuroligin synaptic adhesion molecules contribute to GABA-mediated regulation of inhibitory synapse development. We found that developing basket cell axons and presynaptic boutons are highly dynamic. Interfering with $GABA_B$ receptor func-

tion by either antagonists or single-cell knockouts destabilizes nascent GABAergic terminals. These results suggest that local activation of presynaptic $GABA_B$ receptors stabilizes nascent synaptic contacts and contributes to activity-dependent regulation of inhibitory synapse development. We further examined the role of NRXs by live imaging of NRX–synaptopHlorin (SEP) fusion proteins in basket interneurons and showed that (1) the two major isoforms NRX1 have striking differences in subaxonal localization, whereas the long NRX1α is diffuse along axons with enrichment at presynaptic bouton and the short NRX1β is exclusive to developing presynaptic terminals; (2) the strict presynaptic localization of NRX1β is likely achieved by binding to postsynaptic neuroligins; (3) both NRX1α-SEP and NRX1β-SEP are highly dynamic along basket axons, as revealed by FRAP (fluorescence recovery after photo bleaching); and (4) neural activity and presynaptic $GABA_B$ receptor activation promote the stabilization of NRX1β-SEP at developing presynaptic terminals. Together, these results show how GABA signaling engages the synaptic adhesion system in activity development of inhibitory synapses. Because a prominent role of the fast-spiking basket interneuron network is in organizing the dynamics of neural ensembles, deficient basket cell innervation may have implications in a variety of neurodevelopmental disorders.

Functional Maturation of GABAergic Interneurons in Neocortex

Neuronal circuits often display remarkable plasticity to sensory input especially during early postnatal life. A representative example is that the closure of one eye during a critical period can permanently shift the response property of neurons in the primary visual cortex (V1) to favor inputs from the open eye–ocular dominance (OD) shift. Although much progress has been made in studying the anatomical, physiological, and molecular components of OD plasticity, a comprehensive understanding of how these components are integrated in the context of relevant cortical circuitry remains a challenge. Accumulating evidence suggests that proper function of GABAergic inhibitory neurons in V1 is critical in establishing the physiological circuit architecture that allows OD plasticity to proceed. Indeed, a subset of inhibitory neurons—parvalbumin (Pv)-positive basket cells—is suggested to be involved in OD plasticity, but data so far are correlative.

Combining cell-type-specific Cre knockin mice, Cre-activated viral vectors, and electrophysiology, we systematically examined the developmental changes of the V1 Pv cell network around the critical period (from postnatal 17 to 44), including (1) the intrinsic properties of Pv cells and especially (2) Pv-cell-mediated GABAerigc synapses on both pyramidal cells (Pv-Py) and Pv cells (Pv-Pv). We found that the changes in both intrinsic and firing features of Pv cells support the developmental enhancement of the typical fast-spiking characteristics during this period. As for the Pv-cell-mediated inhibition, both Pv-Pv and Pv-Py synapses exhibit very similar short-term plasticity with slight developmental changes. Interestingly, Pv-Pv synapses always show much faster kinetics than Pv-Py synapses at different development stages. But both types of synapses mature with increasingly faster kinetics with the same developmental trajectory before postnatal 23, and then become stable. This maturation time course correlates with the developmental increase of α1-$GABA_A$ receptors. Such kinetics change is also specific at Pv-cell-mediated synapses. Our results and experimental strategy set the stage for genetic manipulation of specific properties of Pv interneurons to establish a causal relationship between the maturation of the Pv cell network and critical period plasticity in the primary visual cortex.

Cell-type-based Epigenomic Analysis of GABAergic Neurons

A systematic understanding of gene expression profiles and the epigenomes of 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 GABAergic circuits. A major challenge in epigenomic analysis of mammalian brains is that cellular heterogeneity-distinct epigenomes are simply inaccessible to sequencing technology. Using genetic engineering in mice, we are building a comprehensive tagging system that allows purification of chromatin DNA, ncRNA (noncoding RNA), and mRNA from genetically defined cell populations from tissues. Genetic tagging of nucleic acids is achieved by cell-type-specific expression of epitope-tagged DNA- or RNA-binding proteins. DNA or RNA species from a defined cell type are isolated directly from tissue homogenates by affinity purification using antibodies against epitope tags or by fluorescence-assisted cell sorting (FACS) of cells from dissociated tissues. We are analyzing major GABAergic neuron types from several cortical areas and across key stages of cortical development.

GABAergic Interneurons and Neurodevelopmental Disorders

We are studying alterations of GABAergic inhibitory circuits in mouse models of the Rett syndrome (RTT), the best-characterized form of autism spectrum disorder. RTT is 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. We have characterized the maturation of major GABAergic markers in the neocortex of *MeCP2*-null mice before (P14, P21) and after (P30, P60) the onset of their behavior phenotype. Compared to wild type, *MeCP2*-null mice showed *increased* expression of Gad67 and Pv in the cortex at all time points tested. Interestingly, at P14, Pv neurons in the *MeCP2*-null cortex also have more elaborate neuronal processes compared to wild-type sections. These data suggest that *MeCP2* deficiency may *accelerate* the maturation of Pv neurons in the cortex presymptomatically and might contribute to altered development of neural circuits. At P30 and P60, *MeCP2*-null mice showed major deficits of SOM (somatostatin) neurons as indicated by a significant *decrease* in SOM-labeled neurite arbors in the cortex. These data reveal that *MeCP2* deficiency leads to profound and differential alterations in different subsets of GABAergic neurons. These studies will significantly improve our understanding of how altered *MeCP2* expression disturbs the development of GABAergic circuits and reveal new insights into the pathogenesis of RTT.

For the past two decades, the predominant model of *MeCP2* function is DNA binding and transcriptional regulation. In the past year, we have discovered a novel biochemical function of *MeCP2* in regulating *mRNA translation*. We found that *MeCP2* was associated with hundreds of neuronal transcripts in the mouse brain and directly bound subsets of these mRNAs in vitro. In the brains of *MeCP2*-deficient mice, the levels of *MeCP2*-associated mRNAs were not notably altered, whereas the levels of proteins encoded by these mRNAs were significantly altered. Furthermore, we found that *MeCP2* was associated with the translation machinery protein EF1A1 and a negative regulator of translation p54/DDX6. *MeCP2* was detected in polyribosome fractions. Together, these studies reveal an unexpected role of *MeCP2* in mRNA translation and suggest that altered regulation of translation may contribute to the pathogenesis of RTT.

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NEURAL CIRCUIT DYNAMICS UNDERLYING DECISION MAKING

A. Kepecs D. Kvitsiani S. Ranade
A. Lak 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, which 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 in order 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)]

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 a variant 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 after correct choices and 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 rats are willing to wait at the reward port reflects decision uncertainty. Therefore, this task allows us to examine how the timing of the decision to leave the reward port ("abort decision") depends on the uncertainty about the original decision. This will enable us to study how access to an internal measure of decision confidence is used in an adaptive behavior and examine the nature of the neural processes underlying decision confidence.

Orbitofrontal Cortex Inactivation Impairs Confidence Judgments

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 pur-

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

We hypothesized that inactivating OFC leaves the odor-guided decision intact while impairing the use of confidence-guided abort decisions. Therefore, we bilaterally implanted double cannulae to temporary blockade neural activity in the lateral and ventrolateral portions of 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 waiting time patterns of OFC-inactivated animals are disrupted without affecting olfactory discrimination performance.

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 OFC we are investigating in rats are conserved across species. By establishing that 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.

How Uncertainty Boosts Learning

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 data points 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 our odor-mixture categorization task in 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 were dynamically adjusting 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.

Multiple-choice Decision Tasks in Head-fixed Mice

J. Sanders

Systems neuroscience is increasingly relying on transgenic mice to gain molecular control of neural circuits, but the development of well-controlled behavioral tasks that mice can perform has lagged behind. Primate electrophysiologists work almost exclusively with head-fixed animals due to the superior behavioral control available. Head-fixed mouse behaviors would also facilitate the use of large-scale electrophysiological recordings, optical imaging, and stimulation methods that are difficult to implement in freely moving animals. Previous studies of head-fixed mouse behaviors have been restricted to virtual navigation and to single a lick-port to register "lick" or "lick-withheld" responses. These behavioral setups do not allow well-controlled cognitive tasks and therefore we have embarked on the development of response interfaces that would allow mice to make multiple-choice decisions. Therefore, we designed an ultra-light trackball interface, which mice can use to register multiple behavioral responses with their paws. Initial studies using this setup showed that mice can perform at more than 95% accuracy on a two-choice discrimination task requiring them to roll the ball toward the source of a sound for reward. We are currently optimizing our training protocol, as well as developing force-sensitive resistor technology, to reliably register the delicate paw presses of mice in order to create an alternative, potentially easier-to-use multiple-choice interface.

Contextual Novelty Processing in the Mouse Hippocampus

J. Sanders

When experiencing a known pattern of events, we can effortlessly sense when something new and unexpected replaces the familiar. During our earlier experiments on sequence processing, we gathered preliminary evidence that hippocampal cells have a characteristic response to such novel events.

We trained head-fixed mice to listen while an unchanging sequence of eight unique sounds was played, and to respond with a lick upon its completion for reward. We then recorded populations of cells in the CA1 field of the mouse hippocampus and presented randomly interleaved trials containing the familiar sequence, a sequence of totally novel sounds, and a version of the familiar sequence with two sounds borrowed from the totally novel sequence. In this final type of sequence, CA1 cells fired more spikes in response to a novel sound when presented as an unexpected part of the familiar sequence than when the same sound was presented as part of the totally novel sequence. In addition, spiking responses to familiar sounds following an unexpected novel sound were suppressed with respect to the same sounds as part of the familiar sequence.

Most recordings from rodent hippocampus conducted in freely moving animals indicated that neurons behave as "place cells" representing unique spatial locations in the environment. By showing that the same neurons can also signal abstract novelty information, we hope to broaden the classic interpretation of rodent hippocampal processing.

Neural Circuit Dynamics of Genetically Identified Neurons in Behaving Mice

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

Neural circuits exhibit exquisitely precise connections between specific, often molecularly defined, neural cell types. Genetically targeted fluorescent markers provide a powerful handle to explore the specificity in the architecture of neural circuits, mostly using in vitro preparations. Although systems neuroscience has been very successful in relating activity of neurons to perception and behavior, identity of the recorded neurons remains mostly unknown. Our goal is to develop a method for the control and identification of extracellularly recorded neurons in freely behaving animals.

Using channel rhodopsin-2 (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. To activate molecularly defined classes of neurons, we used a knockin mouse line targeting Cre expression to parvalbumin (PV)-type interneurons and generated a new Cre line for calretinin-expressing neurons. To express ChR2, we created a cytomegalovirus (CMV) loxpSTOPloxp-ChR2 adeno-associated virus (AAV) vector, which provides high-level and very specific (>95%) expression of ChR2 in PV^+ interneurons. We designed a new "microdrive" that can house up to 10 independently movable electrodes and/or fiber-optics. 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 electrophysiology record them at the same time in freely moving mice.

We aim to use these tools to understand how the diversity of inhibitory neuron types in medial prefrontal cortex (mPFC) underlies distinct neural circuit dynamics. Specifically, mPFC activity is 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 (4–10 Hz) rhythm (because they can directly inhibit excitatory principal cells), whereas CR interneurons regulate the γ (40–100 Hz) rhythm (because they tend to inhibit other interneurons). Therefore, CR and PV interneurons might have complementary roles in controlling neural population rhythms. Currently, we are testing the functional roles of these interneuron classes by characterizing their activity using optical tagging and stimulating them to observe the consequences on global neural activity and behavior.

Paying Attention to Light: Optogenetic Dissection of Cholinergic System during Behavior

S. Ranade, M. Grabois, J. Sanders

The cholinergic basal forebrain (CBF) is a vitally important yet poorly understood neuromodulatory system that is thought to have significant roles in cognitive functions.

Projections of CBF innervate the entire cortical mantle and release acetylcholine, which modulates cortical processing and enhances synaptic plasticity. Past research, using primarily pharmacology and lesion studies, has identified the neurotransmitter acetylcholine as a key modulator of learning, memory, and attention—key features of cognition. However, the anatomical and neurochemical heterogeneity of the cholinergic system, combined with lack of sophisticated techniques, has prevented a systems-level understanding of the role of acetylcholine in cognitive processes. The goal of this project is to understand the function of the CBF, the neural system underlying the release of acetylcholine, using a powerful combination of molecular genetic, electrophysiological, optogenetic, and psychophysical techniques.

We use a knockin mouse line expressing the Cre recombinase under the control of a cholinergic neuron-specific promoter, choline acetyl transferase, to specifically target cholinergic neurons among the heterogeneous neuronal types of the CBF. Expression of the optical activator ChR2, delivered locally to the CBF via viral vectors, enables us to directly and specifically manipulate cholinergic neurons with high spatiotemporal precision. In addition, we have also successfully developed a sustained attention task in mice. In this task, mice are trained to respond to a target sound and ignore a distractor sound. Mice learn this task in a couple of weeks and show stable performance.

Using these techniques, we are now in position to manipulate the activity of cholinergic neurons during the execution of various cognitive tasks and tease out the role of acetylcholine during behavior. These tools enable an unprecedented degree of mechanistic investigation of the behavioral functions of the CBF system and provide an entry point for future studies of cholinergic degenerative diseases, such as Alzheimer's disease.

Cholinergic Modulation of Auditory Cortical Responses

S. Ranade, T. Hromadka [in collaboration with A. Zador, Cold Spring Harbor Laboratory]

Acetylcholine has profound physiological effects on the activity of its target neurons. For instance, nucleus basalis stimulation leads to desynchronization of the cortical EEG, whereas local pharmacological application of acetylcholine to pyramidal neurons leads to increases in the firing rate, as well as changes in the receptive field properties of cortical neurons. However, most of these effects have been observed with extracellular stimulation and pharmacological manipulations, both of which are susceptible to noncholinergic effects. The goal of this project is to understand the effects of acetylcholine release on spontaneous, as well as stimulus-evoked, activity on cortical neurons.

We therefore targeted the light activator ChR2 to cholinergic neurons (see previous section) projecting to auditory cortex to evoke light-triggered release of acetylcholine. Using this technique, we performed extracellular and cell-attached recordings in anesthetized mice and observed the effect of acetylcholine on auditory cortical responses. We found that a high percentage of neurons increased their firing rate in response to light activation. In some cases, we also observed a broadening and a reorganization of the auditory tuning curves of cortical neurons. These experiments provide a proof of principle that light activation of cholinergic terminals can lead to changes in neuronal responses and provide the foundation for biophysical studies probing the underlying modulatory mechanisms.

Rapid Light-induced Transcription in Mammalian Cells

A. Kepecs [In collaboration with F. Albeanu, Cold Spring Harbor Laboratory]

Genetically encoded optical modulators have revolutionized neuroscience. A serious impediment to their use in probing neural circuitry during behavior is that at present we cannot target them to functionally defined neural populations—only to neurons defined by genetic markers or anatomical projections. One way to achieve functional targeting is to first use imaging techniques to characterize a functional population of neurons and then use light-activated transcription to induce genetically encoded activity indicators and optical modulators specifically in this functionally defined neuron class.

Toward this goal, we are developing a genetically encoded light-induced transcription system that can be used in the brain in vivo. We are presently testing a two-component system that upon light activation would rapidly and persistently release a tTa driver to induce the transcription of any construct under the tTA-responsive promoter tetO. In principle, this system is generic, and therefore, depending on its efficiency and leak, we foresee applications beyond neuroscience for precise spatiotemporal targeting of gene expression.

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A. Kepecs

THEORETICAL AND COMPUTATIONAL NEUROSCIENCE

A. Koulakov D. Tsigankov

Our laboratory develops theoretical models for neural processing. We work in parallel on three important topics. First, we have proposed a model for combining genetic information and experience (nature and nurture) during the development of neural connectivity. Our model describes how genes can help build neural networks and how neural activity adds a layer of plasticity to the network topology that reflects learning and experience. This model has been tested on simple circuits that are formed in the visual system and can be rewired using genetic, surgical, and pharmacological manipulations. Second, we have been developing the neural network theory for olfactory processing. Our theory attempts to describe the olfactory space, which has been an illusive concept taunting the imagination of chemists, neuroscientists, and experimental psychologists alike. In our theory, we attempted to link several levels of olfactory perception: chemical, genetic, neural, and perceptual. Our main result so far has been the mapping between chemical and perceptual spaces that was established on the basis of a database of human olfactory responses. We can say now that we have a robust description of the human perceptual olfactory space. This description is analogous to the understanding of, for example, the main directions in the human color space, i.e., red, green, and blue. These main perceptual directions can be understood in terms of the properties of the underlying chemical compounds, at least, on the level of correlation. We also proposed a network theory for information processing in the olfactory bulb. We suggest that the granule cells, the inhibitory neurons of the olfactory bulb, form representations of smells using the network implementation of sparse overcomplete representations. Granule cells are remarkable because, unlike most of the neurons in the adult mammalian nervous system, they are continuously replaced by the new neurons produced from the neural stem cells. Third, we have been working on the mathematical description of adult neural stem cell differentiation and proliferation in the 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.

Competition Is a Driving Force in Topographic Mapping

A. Koulakov [in collaboration with C. Pfeiffenberger, J.W. Triplett, J. Yamada, B.K. Stafford, and D. Feldheim, Univrsity of California, Santa Cruz]

Connectivity in the brain is established on the basis of chemical labels and structured activity. For the two-dimensional, topographically organized projection from the retina to the superior colliculus (SC), the Eph family of receptor tyrosine kinases and their ephrin ligands exert the role of chemical labels. Despite specific effects of receptor activation on retinal axons, axons respond to relative, rather than absolute, levels of chemical labels, which endows the mapping mechanism with substantial robustness. However, it is not known how axons compare global levels of chemical labels. Here, we present experimental and computational evidence that interaxon competition has a nonredundant role as a mapping mechanism for the retinocollicular projection. We investigated topographic maps in the SC of Math5 mutant mice in which the overall number of retinal axons is severely reduced. We showed that topographic maps are substantially distorted under conditions of reduced competition (Fig. 1). RGC axons project almost entirely to the anterior-medial portion of the SC where repulsion from ephrin-A ligands is minimized and where their attraction to ephrin-Bs is maximized. We conclude that chemical labels alone do not instruct the formation of the map. We provide a mathematical model for topographic mapping that incorporates molecular labels, neural activity, and axon competition.

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 us to monitor the axon, dendrite, and synapse dynamics in vivo. It is observed

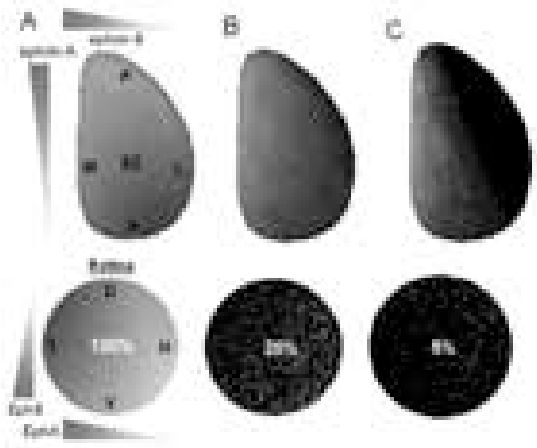

Figure 1. As the number of retinal cells is progressively decreased in the model, the projection from retina to the superior colliculus (SC) of the midbrain deforms until only a part of the target is filled with axons (*top right*). The case of ~5% of retinal cells present corresponds to the phenotype observed in Math5$^{-/-}$ knockout mice, in which retinal progenitors produce only 5% of the normal number of neurons. The structure of retinocollicular connectivity observed in these animals is consistent with the results of the model shown here (*top right*). This argues strongly in favor of the mechanism involving competition between axons for space and trophic factors in the target. Chemical labels alone (Eph receptors and ephrins) cannot enforce the correct connectivity.

that the majority of axon and dendrite branches formed are retracted later during the development. In this study, we computationally analyzed 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 pre- and postsynaptic electric activity. We show that experimentally observed different reactions to the NMDA (*N*-methyl-D-aspartate) 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 NMDA receptor block.

The Structure of Human Olfactory Space

A. Koulakov [in collaboration with A. Enikolopov 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 odorants in this perceptual space. We argue that odorants densely sample a two-dimensional curved surface embedded in the multidimensional sensory space (Fig. 2). This surface can account for more than half of the variance of the psychophysical data. We also show that only 12% of experimental variance cannot be explained by curved surfaces of substantially small dimensionality (<10). We suggest that these curved manifolds represent the relevant spaces sampled by the human olfactory system, thereby providing surrogates for olfactory sensory space. For the case of two-dimensional approximation, we relate the two parameters on the curved surface to the physicochemical parameters of odorant molecules. We show that one of the dimensions is related to eigenvalues of molecules' connectivity matrix, whereas the other is correlated with measures of molecules' polarity. We discuss the behavioral significance of these findings.

Figure 2. The 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.

Sparse Incomplete Representations: A Novel Role for Olfactory Granule Cells

A. Koulakov [in collaboration with D. Rinberg, HHMI Janelia Farm]

Mitral cells of the olfactory bulb form sparse representations of odorants and transmit this information to the cortex. The olfactory code carried by the mitral cells is sparser than the inputs they receive. In this study, we analyzed the mechanisms and functional significance of sparse olfactory codes. We consider a model of the olfactory bulb containing populations of excitatory mitral and inhibitory granule cells. We argue that sparse codes may emerge as a result of self organization in the network leading to the precise balance between mitral cells' excitatory inputs and inhibition provided by the granule cells. We propose a novel role for the olfactory granule cells. We show that these cells can build representations of odorant stimuli that are not fully accurate. Due to the incompleteness in granule cell representation, the exact excitation-inhibition balance is only established by some mitral cells leading to sparse responses. Our model suggests a functional significance of the dendrodendritic synapses that mediate interactions between mitral and granule cells. The model accounts for the sparse olfactory code in the steady state and predicts that transient dynamics may be less sparse.

Disposable Tissue Hypothesis: A New Model for Hippocampal Neurogenesis

A. Koulakov [in collaboration with G. Enikolopov and J. Encinas, Cold Spring Harbor Laboratory]

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., leaves the pool of neuroprogenitors. Therefore, one can think of the stem cells as disposable: Once they are used, they cannot be reused again. The decline in the number of stem cells therefore occurs in a use-dependent manner. Does this mean that the more neurons 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 occurring in the neurogenesis cascade due to antidepressant drugs, aging, and other therapies. Our approach will provide unique insights to the computational properties of the neural stem cells and their importance for mental health.

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FUNCTION AND PLASTICITY OF CENTRAL SYNAPSES IN ANIMAL MODELS OF PSYCHIATRIC DISORDERS

B. Li S. Ahrens
S. Liu
Z. Perova

Research in my laboratory is directed toward understanding the synaptic mechanisms of psychiatric disorders. Synaptic plasticity is believed to serve as the cellular mechanism for learning and memory, and impairments in this process have been linked to psychiatric disorders, including schizophrenia and depression. We use rodent models of psychiatric disorders, as well as a number of complementary methodologies, including behavioral assays, electrophysiology, two-photon imaging, in vivo circuit tracing, electrical deep-brain stimulation, molecular genetics, and optogenetic techniques to address questions in three major areas: (1) the synaptic mechanisms of depression, (2) the synaptic mechanisms underlying normal adaptive behaviors, such as resilience to depression or behavioral flexibility, and (3) the synaptic mechanisms of schizophrenia.

The Synaptic Circuitry of the Lateral Habenula and Behavioral Depression

The neural basis of mood disorders is poorly understood. As a consequence, efforts to develop novel and more effective therapies for depression have largely been unsuccessful. Recent studies indicate that neurons in the lateral habenula (LHb) signal "disappointment" and may have an important role in depression; however, the mechanisms by which the LHb contributes to depression are unknown. We will be testing the hypothesis that aberrant activity in the synaptic circuitry of the LHb underlies behavioral depression and that normalization of LHb neuronal activity is integral to the efficacy of antidepression treatments. The overall goal of this project is to gain a better understanding of the cellular changes responsible for the pathogenesis of depression. Using animal models of depression, we will assess aberrant neuronal activity in the LHb and determine its underlying synaptic mechanisms. Furthermore, by selectively manipulating the activity of LHb neurons and determining the behavioral outcomes, we will be able to test the causal relationship between aberrant LHb neuronal activity and behavioral depression. Importantly, this research program will result in the development of methods that allow the modulation of depression-like behavior in animals.

Depression and Resilience: The Role of the Medial Prefrontal Cortex

We will be testing the hypothesis that normal synaptic plasticity in neurons of the medial prefrontal cortex (mPFC) has an important role in behavioral flexibility and resilience to depression, whereas its impairments lead to behavioral depression. The mPFC has been implicated in executive function and behavioral flexibility. Recent studies suggest that mPFC is critical in the determination of whether an organism has control over a stressor. Rats with inactivated mPFC are prone to behavioral depression induced by stressors (Amat et al., *Nat Neurosci 8*, 365–371 [2005]). Aberrant activity of the mPFC also accompanies clinical depression, and deep-brain stimulation in mPFC leads to remission of treatment-resistant depression (Mayberg et al., *Neuron 45,* 651–660 [2005]). However, the cellular processes in mPFC accompanying behavioral depression are not clear. The cellular mechanisms in mPFC that underlie controllability, therefore affording resilience to behavioral depression, are also unknown. In this research project, we will determine the causal relationship between plastic changes in the glutamatergic synapses onto mPFC neurons and an animal's susceptibility or resilience to behavioral depression.

The mPFC and LHb are synaptically connected and belong to a synaptic circuitry strongly implicated in depression. The first and second projects are integral components of a synergistic effort aimed for a more complete understanding of the synaptic and circuitry mechanisms underlying depression.

Genetic Deficiency, NMDAR Hypofunction, and Schizophrenia

In this project, we are studying the genetic causes of *N*-methyl-D-aspartate receptor (NMDAR) hypofunction,

a pathological process believed to contribute to the etiology of schizophrenia. We will first focus on *ErbB4*, a gene with polymorphisms and mutations associated with schizophrenia. The central hypothesis to be tested is that ErbB4 controls glutamatergic synapse development and plasticity in GABAergic inhibitory interneurons. ErbB4 deficiency early in life will lead to NMDAR hypofunction and impede the recruitment of GABAergic interneurons into cortical or subcortical microcircuits. Furthermore, we hypothesize that this will result in specific circuit dysfunction and aberrant behavior later in life that are similar to those brought about by psychotomimetic NMDAR blockade or by direct NMDAR deficiency. By using a number of complementary techniques including electrophysiology, two-photon imaging, and cutting-edge molecular genetic technology, we will be able to directly test the causal links among genetic deficiency, neurodevelopment deficits, and NMDAR hypofunction, major effectors believed to contribute to the pathophysiology of schizophrenia.

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Bo Li

INTEGRATIVE SYSTEMS NEUROBIOLOGY

P. Mitra P. Andrews H. Cox P. Grange J. Jones J. Kulkarni V. Pinskiy D. Valente H. Wang 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 neurobiological questions. 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 infrastructures 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 has previously consisted of collaborative studies in multiple species including *Drosophila*, zebra finch, macaque monkey, and human infants. We have now started a major in-house experimental project to generate the first brain-wide mesoscale connectivity map in the mouse.

Continuing in our laboratory in 2009 were Peter Andrews (scientific informatics manager), Jayant Kulkarni (postdoctoral fellow), Vadim Pinskiy (graduate student), Dan Valente (postdoctoral fellow), Haibin Wang (postdoctoral fellow), and Caizhi Wu (postdoctoral fellow). Hilary Cox (Research Associate), Pascal Grange (postdoctoral fellow), and Jamie Jones (Laboratory Technician) joined us this past year, and Jason Bohland left our lab to become an assistant professor at Boston University.

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 College of Cornell University, where Dr. Mitra is an adjunct associate professor; a consortium of zebra finch researchers; Josh Dubnau at CSHL with whom we perform an integrative analysis of memory formation in *Drosophila*; the Brain Architecture Project, with collaborators at multiple institutions, notably, Harvey Karten (University of California, San Diego) and Michael Hawrylycz at the Allen Institute of Brain Research; and a collaboration with Gregory Hannon at CSHL on improving next-generation sequencing technologies.

The Brain Architecture Project

H. Cox, V. Pinskiy, C. Wu, P. Grange, H. Wang, J. Jones

In a famous article in the early 1990s, Francis Crick and Ted Jones bemoaned "the backwardness of human neuroanatomy." After almost a century of modern neuroscience and the Decade of the Brain, we still know shockingly little about how our own brains are connected. We have set out to address this problem in the Brain Architecture Project, starting with the mouse and ultimately working toward the human brain.

The problem we address is fundamental. Brain function is dictated by its circuitry, but currently, we know comparatively little about how the mammalian brain is connected: Only 10% of all mesoscale connections in the most-studied mammal (rat) have been cataloged and only perhaps one-third have been probed at all.

In a position paper published in *PLoS Computational Biology* in 2009, we proposed a large-scale project to determine the brain-wide interregional connectivity matrix of the mouse brain at a mesoscopic resolution (see Fig. 1). To execute these plans, we have successfully obtained two National Institutes of Health (NIH) grants this year (a Transformative-R01 an RC1 Challenge Grant).

A high-throughput pipeline has been developed to process tissue from tracer-injected mice through to image analysis. For each injected location, after 3–21 days of tracer transport, the whole brain is sectioned and imaged using an automated microscope system. The software components of the pipeline take these stacks of digitized microscopy images and perform segmentation and three-dimensional reconstruction to reconstitute the three-dimensional brain. This brain volume is then registered with the Allen Reference Atlas (ARA) in a series of steps. These registrations allow us to determine for each tracer injection where the injected area projects to and from. By combining the data from across the full cohort of 200–400 injection sites, a complete wiring diagram can be generated.

Although the total amount of data generated by the project is expected to be large (terabytes to petabytes), we expect that the principal challenges will be in terms of

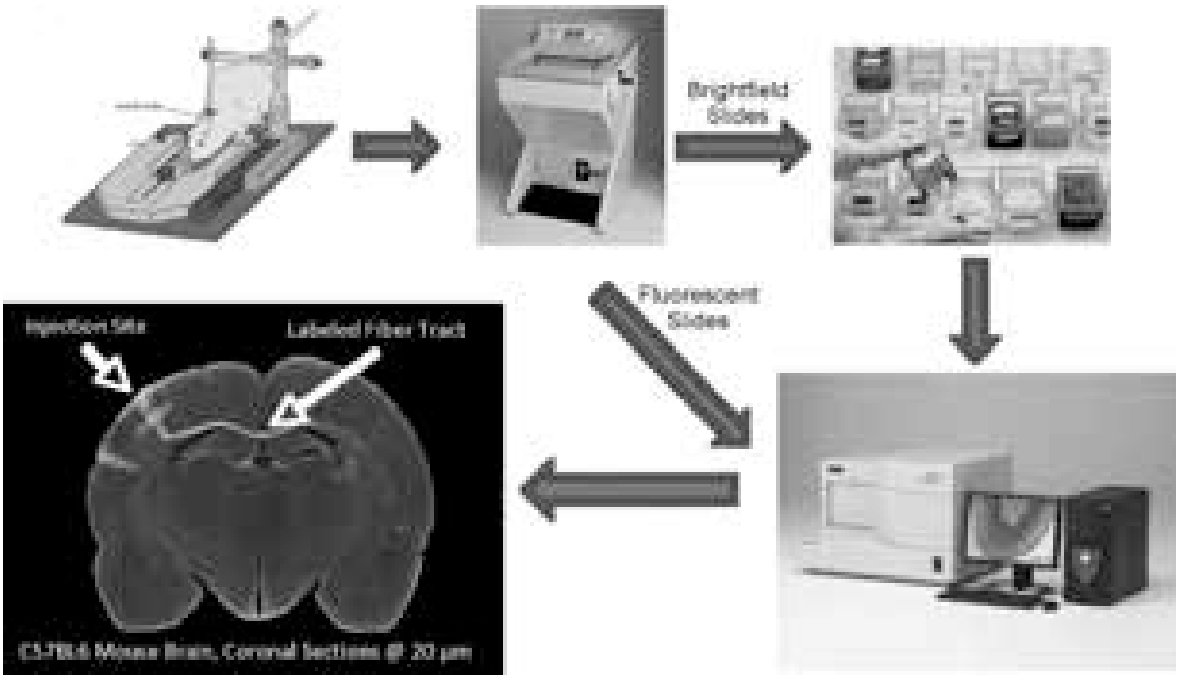

Figure 1. Stages of the proposed pipeline for the "connectivity scanner." The images indicate the major experi-

software to enable suitable compression schemes to permit effective transmission through the Internet (which remains a low-bandwidth medium), as well as suitable visualization and search tools. The Allen Institute has extensive experience in this domain, and Dr. Mitra has established a collaborative relationship with Dr. Mike Hawrylycz at the Allen Institute. He will work with the advice of Dr. Hawrylycz to ensure that the informatics infrastructure works across time in the manner specified.

The availability of public data sets and open source duplicatable pipeline technologies will potentially impact the entire neuroscience community and the agendas of at least eight of the 20 NIH institutes. Generating the first unbiased, brain-wide connectivity map in the mouse brain will have broad neuroscientific implications. The study of neural development, associative and integrative brain function, and brain evolution will benefit tremendously from finally having the data necessary to meaningfully constrain theories. We also propose to demonstrate the disease-model utility of this approach in order to hasten its widespread adoption. We have chosen autism and schizophrenia because they are among the most heritable, prevalent, and devastating neuropsychiatric disorders afflicting humanity.

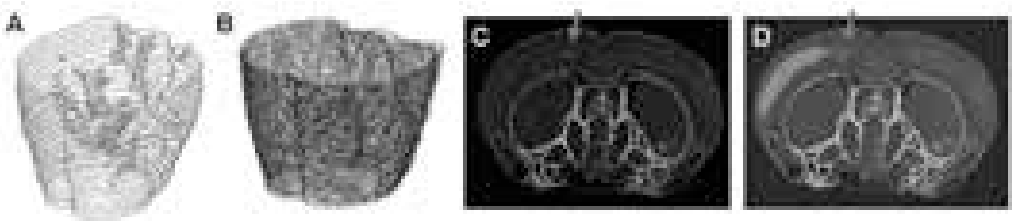

Figure 2. Automated registration of reconstructed section data to ARA. (*A*) Stacked outer contours of reconstructed sections. (*B*) Affine registration with outer contours of ARA using Hausdorff distance measure. (*C*) Two-dimensional deformable B-spline registration of ARA to a two-dimensional section, showing cortical parcellation (*left*). Tracer labeled cells (arrows) are localized within the primary motor cortex. (*D*) Nissl labeling registered to ARA with overlay of expression of the *LOC4332228* expression pattern, highly expressed in Layer 4, demonstrating integration with the Allen Brain Atlas.

We are additionally developing a novel technique for assaying long-range connectivity in the human brain and are pursuing the aims of mining and integrating information about brain connectivity from the research literature using computational linguistics techniques.

Through this project, Cold Spring Harbor Laboratory has become a leading center for filling the critical knowledge gap urged by Dr. Crick, and championed by Jim Watson.

For more information, see the Brain Architecture Project website: http://brainarchitecture.org.

Gene Expression in the Mouse

P. Grange

We continue our collaboration with the Allen Institute of Brain Science with a project to leverage the genome-scale high-resolution expression maps created within the Allen Brain Atlas (ABA) to study coexpression patterns of genes implicated in addiction and related phenotypes. Pascal Grange has just joined Dr. Mitra's lab as a postdoctoral fellow and will be working closely with the Allen Institute. Pascal was previously working on string theory at the Institute for Advanced Study at Princeton and will be analyzing the Allen Institute data set of genes expressed in the mouse brain, using advanced statistical methods.

Toward an Integrative Model of Memory in *Drosophila*

D. Valente

In 2009, Dr. Mitra's professorship supported a project aimed toward an integrative model of memory formation in *Drosophila* (postdoctoral fellow: Dan Valente). During the past year, we have continued our behavioral studies of flies in a flight simulator, an assay that will allow us to dynamically probe the learning process at all levels of biological organization in a single animal. Dr. Valente has made significant improvements to the training protocol and has performed a series of experiments aimed at understanding the range of system parameters required to observe learning in this setup. A precise description of these parameters has been essential in generating hypotheses regarding fly nervous system mechanisms. Dr. Valente has also begun work on a predictive model for flight behavior. This model will allow us to better match the virtual flight experience to a real flight experience and maximize the ability of flies to form associative memories. The success of this project will establish a single-fly visual learning assay for use by all the fly labs at CSHL and will provide the *Drosophila* learning community with a more detailed description of the dynamics of the learning process.

We also continued our collaboration with Josh Dubnau's lab on an experimental evolution study of epistatic gene interactions in *Drosophila*. Our role has been the analysis of genotypic results at two generations of the selective breeding. We have developed a method that uses multivariate analysis and machine learning techniques to predict alleles responsible for the phenotypic difference between selected and unselected groups. These predictions were then experimentally verified in the Dubnau lab. This work is being prepared for publication.

Advanced Algorithms for Genetic Sequence Analysis

D. Valente [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.

We are currently working on a method to improve base calling in the next-generation sequencing platform from Illumina. This started as a collaborative project with Greg Hannon's lab at CSHL. We have extended our previously published random-walk model which describes phasing and fading errors to include cross-talk errors during the sequencing process. Dr. Valente is currently writing a base caller using this extended model, which is expected to be a vast improvement over current base-calling techniques. The algorithm is near completion and the work is being prepared for publication.

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, to develop signal processing algorithms, and to analyze experimental data from collaborating laboratories.

We proposed a theoretical framework to understand how the song culture evolves, through interactions among phenotype, genotype, and environment. Our models illustrate 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.

The publication this year of a manuscript in *Nature,* "De novo emergence of song culture in the zebra finch," is the culmination of several years of collaborative work with Ofer Tchernichovski's lab at City College of New York. This research dealt with the complex interrelationship among nature, nurture, and culture in the tractable animal model provided by the song system in the zebra finch. Songbirds exhibit social learning of song vocalizations, and song culture provides a laboratory-like microcosm with salient similarities to human culture. Birdsong research has traditionally focused on the learning of a song by the juvenile from adults, but this research deprecates the origin of the adult cultured song.

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, which are available at www.zebrafinch.org/atlas as part of our informatics infrastructure for zebra finch birdsong study.

Meetings/Training/Education

P. Mitra

The first meeting for the Mouse Brain Architecture Project (MBAP) was held December 1, 2009 at Cold Spring Harbor Laboratory. The meeting was attended by 45 individuals, including project staff, collaborators, scientists conducting related research, representatives from relevant vendors, and representatives from the NIMH (National Institute of Mental Health). MBAP staff members introduced the project, described advancements made in terms of tissue sectioning protocols, and sought expert opinions from meeting attendees on the experimental choices that will be made within the pilot phase of the project. Meeting attendees also gave short presentations describing related neuroanatomy projects in mouse and other species and about issues related to microscopy and informatics. Finally, there was a discussion about the development of an upcoming CSHL summer course in modern neuroanatomy, and an art exhibit featuring sculptor Fré Ilgen's rendition of the nervous system.

Jason Bohland completed his postdoctoral fellowship in my laboratory and has earned a faculty position at Boston University to continue our work as collaborators.

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NEURAL ENDOPHENOTYPES IN MOUSE MODELS OF AUTISM AND SCHIZOPHRENIA

P. Osten Y. Bao N. Takada
L. Kadiri J. Taranda

What causes autism and schizophrenia? It is well established that both psychiatric disorders are highly heritable and human geneticists are uncovering the underlying susceptibility genes. The growing wealth of genetic knowledge, however, does not guarantee rapid progress in understanding what causes autism and schizophrenia at the level of neural circuits. In fact, even though it is generally believed that these disorders are caused by subtle disruptions in brain circuits, our understanding of which particular circuits are affected is rudimentary at best.

Our research focuses on the study of neural circuits in genetic mouse models of autism and schizophrenia. We have established a novel high-throughput microscopy method, which enables us to rapidly map mouse brain circuits at cellular resolution. We are now applying this method to systematically compare brain circuits in different genetic mouse models, with the aim to begin mapping the causal relationship: *Genome→ neural endophenotype→disease manifestation*. The experimental questions we ask are:

1. What neural circuit deficits are linked to specific genetic mutations? This research is done by comparing neural circuits between mutant mice and wild-type littermates, with the aim of identifying gene-specific neural endophenotypes.
2. How can many genetic variants result in a similar disease manifestation? This research is done by comparing different genetic mouse models, with the aim of identifying shared "core" neural circuit endophenotypes.

Our work uses whole-mount microscopy, transgenic reporter mice, anatomical tracing, and electrophysiology. Below we describe our progress in these areas.

Whole-mount Microscopy

Two-photon microscopy for automated whole-mount imaging is a key part of our experimental approach (Fig. 1). The microscope, built in a collaboration with TissueVision Inc. (Cambridge), works as follows: (1) A fixed mouse brain is placed on a motorized stage, which moves it in *x-y* direction so that the top view is imaged as a mosaic. (2) The stage moves the brain toward a built-in vibratome, which cuts off the imaged section. (3) The cycles of mosaic imaging and sectioning are repeated. Once all data are collected, image-processing and brain-morphing software are used to assemble the brain sections and align them to a virtual brain atlas for data analysis (Fig. 2).

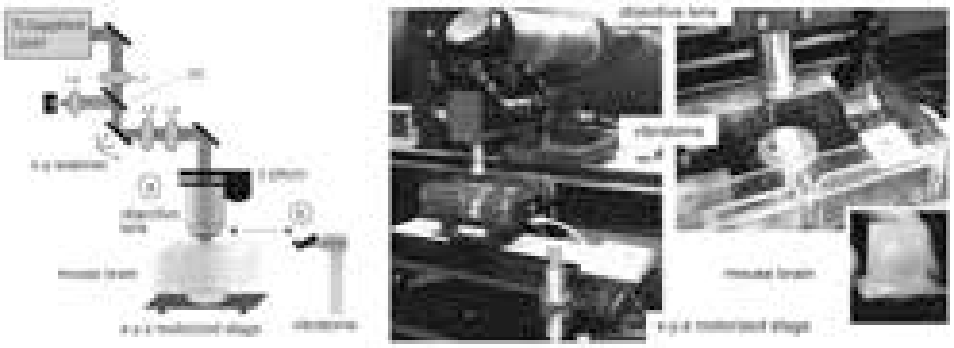

Figure 1. Schema and the actual instrument. The *x-y-z* stage moves the brain under the objective, so that the top view is imaged as a mosaic. The stage also delivers the brain to the vibratome for sectioning.

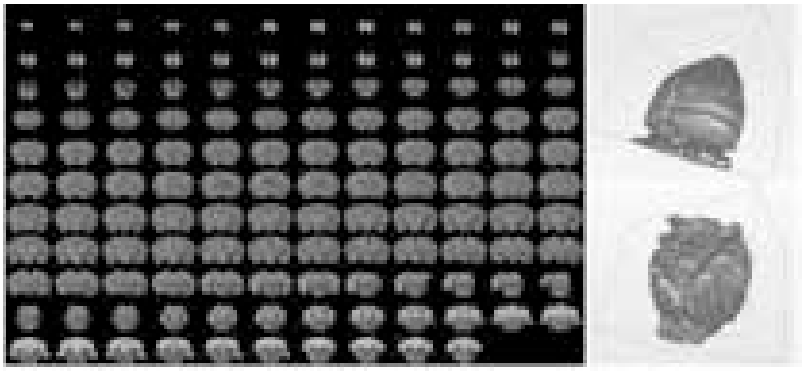

Figure 2. Mouse brain imaged by whole-mount microscopy. Transgenic green fluorescent protein (GFP)-expressing mouse brain was imaged rostrocaudally in 130 sections. The single sections were then realigned in a three-dimensional volume, as shown on the right. The total imaging time was 5 h 49 min.

Transgenic Reporter Mice

To map brain circuits by whole-mount microscopy, we use transgenic reporter mice expressing GFP from promoters of immediate-early genes (IEGs), such as *c-fos* and *Arc*. Because IEG expression is regulated in the brain by neural activity, these reporter mice enable us to monitor IEG expression and by extension neural circuit activity by simple visualization of fluorescent labeling of GFP-positive cells (Fig. 3). Our approach to use this method to screen brain circuits in mouse models of autism and schizophrenia involves breeding selected genetic mouse models with the reporter mice and analysis of neural circuits in the mouse offspring after pharmacological activation of specific brain circuits by neurotransmitter agonists and antagonists (Fig. 4). We have initiated work on three mouse models of autism: the 16p11.2 deletion and duplication mice generated by our collaborator Alea Mills at CSHL and neuroligin R451C mice generated by Tom Südhof's group (Stanford). We first focus on analysis of the balance between excitation and inhibition (E/I) in the brain, testing a hypothesis that autism susceptibility genes cause E/I disbalance during development. We reason that such E/I disbalance may not only increase the risk of seizures as observed in autistic children, but also cause more subtle circuit disruptions contributing to autistic behavior.

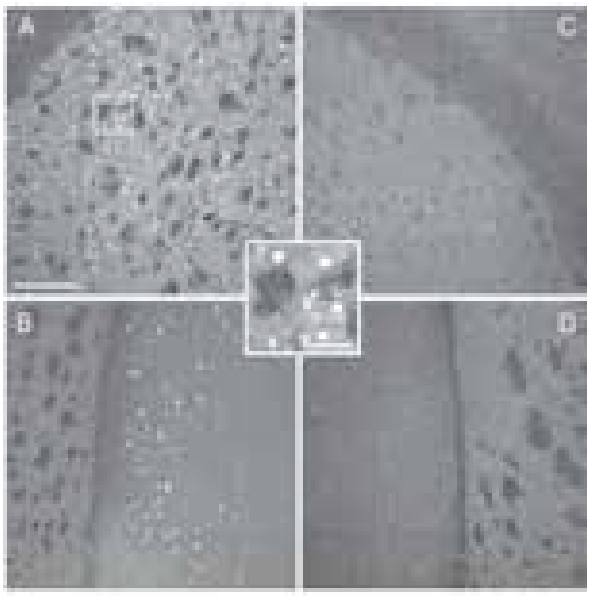

Figure 3. IEG-based visualization of activated neural circuits in the c-*fos*–GFP reporter mouse. Clear GFP labeling is seen in activated neurons in the striatum (*A*) and lateral septum (*B*) after injection of an antipsychotic drug haloperidol (*A–B*), but not in saline-treated control mice (*C–D*). Bars: (*A*) 200 m, (*inset*) 50 m.

Anatomical Tracing and Electrophysiology

The work described above aims to identify brain areas and circuits disrupted in different genetic mouse models of autism and schizophrenia. Once such regions are found, our next step is to use anatomical tracing and electrophysiological recording to examine in detail the anatomical and cellular changes underlying the circuit deficits. Anatomical tracings are done using traditional tracers, such as cholera toxin B for retrograde tracing, and virus vector-based mapping of anterograde axon pathways. Electrophysiological recordings are done in vitro in acute brain sections and in vivo by whole-cell recording in anesthetized mice.

Summary

As our work progresses, we believe that we will be able to begin to "connect the dots" between whole-brain screening of neural circuits and anatomical and electrophysiological analyses of the relevant pathways and

Figure 4. Experimental pipeline. Autism mouse models are bred with IEG-indicator mice and neurotransmitter agonists, and antagonists are used to activate specific brain circuits in the mouse offspring. Behavior (overall activity, seizure induction) is monitored to evaluate the relationship: drug dose→behavioral effect→brain circuit activation.

brain areas. We expect that this work will provide a deeper understanding of neural endophenotypes underlying autism and schizophrenia in humans. Importantly, we hope that our work will also lead to translational opportunities: If our approach allows us to discover signatures of neuropsychiatric disorders in the mouse brain, then it can also be used to search for treatments by screening for drugs that eliminate the abnormality and restore normal activity.

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Julian Taranda, Pavel Osten

RARE STRUCTURAL VARIATION IN THE GENOME AND ITS ROLE IN HUMAN DISEASE

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

The major focus of our laboratory is to identify genetic causes of mental illness using a successful approach that we pioneered: high-resolution 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 the 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. With support from the Stanley Foundation, we have now successfully applied this approach to studies of schizophrenia and bipolar disorder, and our work has already had a significant impact on the field. Our findings, coupled with studies by other groups, have shown that rare CNVs have an important role in the genetics of psychiatric disorders. Furthermore, we have shown that a substantial number of the risk variants detected in the DNA of patients are not inherited from a mother or father but instead occur by spontaneous mutation. 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.

Analysis of Copy-number Variation in Schizophrenia

V. Vacic, S. McCarthy

Studies by our group and others have demonstrated that individually rare structural variants contribute to risk of schizophrenia (SZ) and other neurocognitive disorders. Multiple studies have now shown that the mutational burden of rare structural variants is significantly greater in patients with SZ than in healthy controls. In addition, specific loci have been identified where structural mutations are significantly associated with SZ. For example, large deletions at 1q21.1, 15q13.3, and 22q11.2 have been shown to confer substantial risk of SZ. A recent study by our group has found that microduplications of 16p11.2 are significantly associated with SZ (Fig. 1, Table 1; see McCarthy et al. 2009). The SZ-associated regions described here confer significant risk. For instance, the observed odds ratios for the microduplication of 16p11.2 range from 8.3 to 25.8.

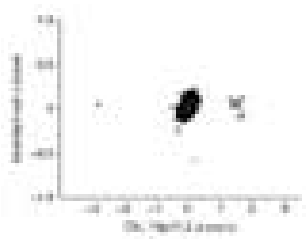

Figure 1. Detection of 16p11.2 microduplications by MeZOD.

These findings suggest that rare structural variants have a role in the etiology of SZ. Furthermore, evidence suggests that little of the overall contribution of rare CNVs to SZ can be explained by the handful of loci described above (Sebat et al. 2009). We hypothesize that genetic risk of SZ consists in part of rare variants, and these risk alleles involve many different genes. Analysis of structural variation in larger samples is required to definitively identify novel CNV risk factors.

In an effort to identify novel CNV risk factors in SZ, we have recently carried out a two-stage study of copy number variation, using a primary sample of 742 cases and 856 controls scanned in the Nimblegen HD2 platform, and using as a replication data set CNV calls made from the GAIN and MGS studies of SZ and the International Schizophrenia Consortium (ISC). The replication sample consisted of 7488 cases and 6689 controls, bringing the combined sample size to 8230 cases and 7545 controls.

Table 1. Duplications and Deletions at 16p11.2 among Persons with Schizophrenia and Controls

		Subjects	Deletions		Duplications			
Series	**Diagnosis**	**(*n*)**	***n***	**%**	***n***	**%**	**OR (95% CI)**	***P* value**
Primary	schizophrenia	1906	1	0.05	12	0.63	24.8 (3.3, 199)	1.2×10^{-5}
	controls	3971	3	0.08	1	0.03		
Replications	schizophrenia	2645	0	0.00	9	0.34	8.4 (1.3, 50.5)	0.022
	controls	2420	1	0.04	1	0.04		
Combined	schizophrenia	4551	1	0.02	21	0.46	14.5 (3.3, 62.0)	4.3×10^{-5}
	controls	6381	4	0.06	2	0.03		

In the first stage, we screened the primary sample for CNVs that are present in multiple cases and not present in a sample of matched controls. After eliminating CNVs that were less than 100 kb in size and CNVs that did not impact genes, we identified a total of 66 unique target loci. To measure statistical association in the replication sample, we tested the difference in locus-based mutational burden between cases and controls using a Fisher's exact conditional test. In this study, all of the previously identified loci described above showed a statistically significant association (data not shown). In addition, duplications at a single 7q36.3 region was significantly associated with SZ after correction for the 66 loci tested ($\alpha = 0.0008$).

The 7q36.3 region represents a novel locus that confers significant risk of SZ. This finding serves to highlight novel genes within the region. One of particular interest is the vasointestinal peptide receptor 2 (VIPR2). VIPR2 is a class II G-protein-coupled receptor that is activated by both vasoactive intestinal peptide (VIP) and pituitary-adenylyl-cyclase-activating polypeptide (PACAP). VIP and PACAP have been shown to have wide pharamacological effects and biological functions. Notably, PACAP is involved in pain-related behavior, psychomotor functioning, and memory performance. PACAP-deficient mice display increased locomotor, exploratory and explosive jumping activity, and a deficit in prepulse inhibition of the acoustic startle response, all of which are attenuated by risperidone. In addition, within the superchiasmatic nucleus, VIPR2 is required for maintenance of normal circadian rhythms.

Investigating the Role of Rare CNVs in Bipolar Disorder

D. Malhotra

Our research in bipolar disorder (BD) aims to make a significant contribution to the field in two ways: by improving the scientific understanding of BD 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 (1) to perform genome-wide analysis of copy-number variation in bipolar families, (2) to assess the overall contribution of de novo and inherited mutations in sporadic and familial BD, and (3) to identify novel candidate genes for further study.

Central to this study is the recruitment of new bipolar families who will form the GEM family collection. GEM is unique from other family collections in that a significant fraction of patients are "sporadic" cases and a significant fraction of patients have an early age at onset. (AAO < 18). This new collection will be made available to the scientific community where we anticipate it will become a valuable resource for researchers who are interested in investigating genetic and epigenetic causes of bipolar disorder.

We have completed a preliminary analysis of data from our genetic study of BD (described in this report), and these data showed that rare structural variants, including de novo mutations and inherited CNVs, contribute to BD. We have performed an analysis of de novo CNVs in families and observed a higher rate of de novo mutation

Table 2. Significant Association of 7q36.3 Microduplications with Schizophrenia

		Primary sample		Replication sample			
Locus	**CNV type**	**cases**	**controls**	**cases**	**controls**	**OR [96% CI]**	**replication *P* value**
7q36.6	duplication	2	0	12	0	INF [3.3, INF]	0.0002
2p16.3/NRXN1	deletion	2	0	14	2	6.4 [p1.7, INF]	0.0022
3q29	duplication	2	0	8	0	INF [2.0, Infinity]	0.0049

in BD compared to healthy controls. Furthermore, we observed a higher rate of de novo mutation in sporadic cases as compared to patients with a family history of mental illness. These results provide strong preliminary evidence that de novo CNVs contribute in part to the genetics of BD. A majority of these mutations involve genes, thus highlighting strong candidate regions for further study. In addition, we have performed an analysis of rare inherited CNVs in families. These results provide preliminary evidence for the increased mutational burden of rare inherited CNVs in BD.

Analysis of Structural Variation in the 1000 Genomes Project

S. Yoon, K. Ye

The primary goal of this project is to define genome-wide patterns of structural variation by high-resolution analysis of CNV using two advanced technologies: microarray comparative genomic hybridization (CGH) and next-generation sequencing (NGS). We have a complete collection of all primary data for the project, including a total of more than 500 HapMap samples. We have begun the process of validating CNVs and genotype calls and have designed a custom Agilent array containing dense coverage of ~800 common CNVs and an additional set of rare inherited and de novo CNVs. Microarray CGH analysis will be performed on HapMap samples using this custom array to validate our CNV calls and to better define the breakpoints of variants identified in this study.

Through our participation in the 1000 genomes project, we have performed an analysis of structural variation in whole-genome sequence data on multiple individuals. For these studies, we developed a novel method for detection of CNVs based on coverage depth: event-wise testing (EWT). This call set is currently being integrated with CNV call sets from multiple groups using complementary approaches. These results will constitute the first official data release of the 1000 genomes pilot project, which will tentatively be published in late fall.

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NEURAL CIRCUITRY FOR SOCIAL COMMUNICATION

S. Shea H. Demmer

The overarching research goal in our laboratory is to understand how processing in specific brain circuits works to support natural communication behaviors. We aim to reveal neural mechanisms that allow organisms to detect and recognize familiar individuals, to gather information about their identity and social status, and to select appropriate behaviors. Mice are capable of acquiring detailed profiles of one another from the smells and sounds experienced during their social encounters. These dossiers may include information on a mouse's sex, genetic identity, reproductive state, levels of distress or sexual interest, or even recently consumed foods, details that are indispensable for survival and mating success. Initially, we are working to understand the neuronal activities and mechanisms in primary sensory brain areas that support these forms of communication. In the future, we anticipate moving deeper into the brain to ascertain where the sensory data from those regions are collected and integrated into hormonal and electrical signals that promote appropriate behavioral choices.

The scientific benefit of this approach is twofold. First, we want to identify fundamental principles for how the brain controls complex behavior. To this end, it is our belief that the nervous system's function is best interpreted in the context of the behaviors it was evolutionarily designed to perform. Thus, it is advantageous to use natural behaviors such as intraspecific communication. Second, impairment of social perception and cognition is a core feature of autism spectrum disorder (ASD); for example, patients may have difficulty perceiving and interpreting communication gestures such as speech, facial expressions, and "body language." This broad feature is recapitulated in many mouse models of ASD that carry genetic variants identified in human ASD populations. Therefore, if we can ascertain the neural circuit substrates of social behavior in normal mice, we can make and test predictions for how the circuitry is affected in the mouse models. The results are likely to tell us more about the synaptic modifications that occur in human autism.

The three broad areas of research in our lab concern (1) olfactory communication and memories for familiar individuals, (2) examination of vocal (auditory) communication between mother mice and their pups, and (3) recording from neurons in awake behaving animals to reveal the neural encoding of social cues during a live encounter with another mouse.

Noradrenaline and Memories for Familiar Individuals

How do we remember individuals that we have previously encountered? Substantial evidence indicates that many animals remember each other based on olfactory cues. Memories are especially strong for individuals encountered during key life events such as mating with a new partner or the birth of a litter of young. These important events typically evoke massive release of the neurochemical noradrenaline (NA), initiating a heightened state of emotion and arousal. This surge appears to cause long-lasting modifications to the responses to odorants in the olfactory bulb, which is the first processing station for scent in the mammalian brain. Indeed, it was hypothesized that the coincidence of an odor stimulus with a surge of NA is minimally sufficient to store a memory. We exploited the intimate relationship between NA, olfactory bulb activity, and behavior to create and study olfactory memories in the anesthetized mouse. We discovered that indeed, when NA release is evoked by stimulating locus coeruleus, the source of most NA, while the sleeping mouse sniffed an odorant, neural responses to that odor underwent specific long-term alterations. Remarkably, once awake, the mouse's subsequent behavior toward the odorant was also changed. In other words, the mouse seemed to remember the odor and treat it as though it were familiar.

The ability to induce ethologically relevant memories under anesthesia opens up exciting possibilities for observing the synaptic mechanisms underpinning such memories using advanced techniques that are currently impossible in behaving animals. For example, we are beginning to use several imaging approaches in genetically modified mice during memory formation. These experiments will allow us to separately visualize olfactory bulb input and output as well as wide-scale neuronal populations to ascertain how and where NA-dependent plas-

ticity is coordinated and interacts among neuronal populations. We are also beginning to use high-resolution electrophysiological techniques targeted to specific cell types in order to build a circuit picture of how different olfactory bulb cell types adapt their firing to result in long-term changes to circuit output. Finally, in collaboration with CSHL colleagues Bo Li and Florin Albeanu, we intend to visualize and record the detailed molecular changes associated with olfactory memories. Most forms of long-term plasticity at excitatory synapses involve the redistribution of receptors for the neurotransmitter glutamate into and out of the synapse. This process can be observed by imaging the movement of receptors tagged with a fluorescent protein. We will use virus-mediated gene delivery to express modified receptors in specific neural populations and then induce olfactory memories in vivo in anesthetized mice to observe the movement of these receptors at the synapse to strengthen neuronal connections that store the memory. This series of experiments, if successful, will provide an unprecedented window onto the molecular underpinnings of a natural form of memory as it is forming.

Vocal Communication between Mothers and Pups

Far outside the range of our hearing, in the ultrasound band, mice are constantly holding conversations with one another in a language that is poorly understood at best. Many types of vocalizations are emitted by males and females, juveniles and adults, in a variety of behavioral contexts. We would like to better understand the perceptual significance of these calls to the mice and how they are used to guide behavioral choices.

One form of vocalization that is actually reasonably well understood is the pup distress call. Before acquiring vision and full mobility, young mice will occasionally become separated from the nest. This is stressful for them and they will therefore call out to their mother with a very high-frequency peep. New mothers develop sensitivity to these cries and respond by moving toward their source (phonotaxis) to retrieve the pup. Mothers will also approach a speaker emitting playback of synthetic calls, providing a simple assay for their perception of manipulated calls. Such experiments suggest that there are sharp limits to the types of sounds that will elicit phonotaxis, possibly implying a neural selectivity mechanism that creates a perceptual boundary between pup distress cries and other sounds and vocalizations. We are using optogenetic techniques to probe inputs to ultrasound-sensitive neurons and hopefully reveal synaptic mechanisms for selective responses to distress calls in the auditory cortex of mothers.

Neural Activity during Social Encounters

We are building a setup for recording individual neurons during social encounters and other behavioral assays involving the perception of social and nonsocial information. There are two broad related goals to this approach. The first goal is to examine the encoding of social information such as body odors and vocalizations in primary sensory structures of awake animals. We hypothesize that activity in response to these signals may be labile to associative learning, attention, and arousal which we may be able to manipulate in the context of social encounters. The second goal is to record from neurons in deep-brain neuromodulatory centers during these encounters as well. Neurons that release noradrenaline and dopamine are likely responsive to social signals and may modulate encoding of sensory data and associative plasticity. Understanding the context-dependent activity patterns of these neurotransmitters is therefore critical to developing models for how they affect behavior.

NEURAL CODING AND MEMORY FORMATION IN THE *DROSOPHILA* OLFACTORY SYSTEM

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

The brain has a tremendous capacity to form many highly accurate memories; it is precisely this facility that is lost in diseases such as Alzheimer's and other dementias. Our overall goal is to understand how brain areas involved in learning and memory represent different stimuli with distinct patterns of neural activity and how these activity patterns are modified by learning.

We use the olfactory system of *Drosophila* as a model for investigating these questions. Our research focuses mostly on a brain area involved in learning and memory known as the mushroom body (MB), which is analogous to the olfactory cortex in the mammalian brain. To understand how olfactory information is represented in the MB, we monitor neural activity using both electrophysiological and functional imaging techniques. We have found that MB neurons are extremely odor-selective in their responses. This high selectivity is a general feature of brain areas involved in learning and memory, including the hippocampus and cerebellum. Using the simplicity and genetic manipulability of *Drosophila*, our goal is to establish how this specificity arises and how it is maintained in the face of learning-related changes. The extremely powerful genetic tools in *Drosophila* enable us to manipulate neural activity in precisely defined ways that help us to understand how this circuit functions. For example, by controlling activity of the neurons immediately presynaptic to the MB, we can determine how MB neurons integrate different inputs and establish how many inputs must be active and with what temporal properties to drive a MB neuron to respond. Using this type of approach, we are currently evaluating how MB response properties arise, and how they are modified by the action of important neuromodulatory systems. Additionally, extensive genetic studies of olfactory learning and memory have identified many of the genes involved in learning and memory. One of our goals is to understand how these molecules affect network-level activity.

Population Coding in the Olfactory System

R. Campbell, K. Honegger

Our goal is to establish how different stimuli are represented in distinct patterns of neural activity—how different do two combinatorial patterns of olfactory receptor neuron (ORN) activity have to be in order to be recognized as distinct? We are assessing whether they are distinct by evaluating population-level responses in MB neurons and by measuring olfactory discrimination using behavioral assays. These experiments will tell us what is the smallest unit of activity that makes two olfactory stimuli distinct.

We chose two different types of olfactory stimuli to evaluate how distinctly they are represented: (1) Of a panel of 110 monomolecular odor compounds, we chose the pair of odors that evoked the most similar response pattern in the ORNs and (2) we used a blend of two odors and varied the relative levels of the two, smoothly morphing the blend from [100%A 0%B] to [0%A 100%B]. To investigate population-level neuronal responses to these stimuli, we track the activity of >100 neurons simultaneously using functional imaging with a genetically encoded calcium indicator. We selectively express this indicator in MB neurons using standard genetic approaches and use two-photon imaging to monitor the activity of ~150 MB neurons simultaneously. Using multivariate analysis techniques, we have established how effectively an ideal observer could discriminate different odors based on the activity patterns in the MB. This gives us a description of how discriminable odors are in terms of neural activity—a "neurometric" curve. We find that the ideal observer can distinguish both the extremely similar pair of monomolecular odors and the odor blend at [70:30] versus [30:70].

Although an ideal observer can distinguish these stimuli, can the fly really achieve this? We have tested this behaviorally using the standard Pavlovian paradigm

of learned olfactory discrimination in a T maze. We find that flies are in fact capable of distinguishing the monomolecular pair of odors. They are also capable of distinguishing the [70:30] versus [30:70] blends, although their performance is lower, indicating that it is a more difficult discrimination. By evaluating finer gradations in the blend odor, we plan to identify the point in the psychometric function where two blend odors are just barely distinguishable. By evaluating the MB odor responses at this point, we will be able to determine the pattern of activity that makes two odors just detectably different from each other. This will establish the smallest unit of activity that distinguishes two olfactory stimuli, a fundamental quantity for understanding how olfactory stimuli are represented in neural activity.

Combinatorial Coding of Olfactory Information

E. Gruntman, K. Honegger, G. Turner

The brain has a limited number of sensory inputs, which must be used combinatorially to endow the brain with its immense representational capacity. This is particularly true in olfactory system: It has been estimated that humans can recognize 10,000 different odors even though we only have ~300 different ORNs. Odors elicit responses in multiple neuron types at the first two layers of the olfactory system, consistent with the combinatorial coding hypothesis. However, for a combinatorial code to be meaningful, there must be downstream neurons that respond selectively to particular combinatorial patterns of input activity. Anatomical evidence suggests that the neurons at the third layer, i.e., the MB, are the neurons that integrate combinatorial information. MB neurons exhibit extremely odor-specific responses. We hypothesize that this selectivity arises because MB neurons receive and require multiple different inputs to be active in order for a MB neuron to exhibit a spiking response.

We are testing this hypothesis in two ways. First, we are using the light-activated ion channel channelrhodopsin to stimulate specific second-order neurons (projection neurons or PNs) while recording synaptic responses in MB cells. By determining the frequency of a synaptic connection between the stimulated PNs and the MB population, we have derived an estimate of the connectivity across these layers. These experiments indicate that MB neurons likely receive inputs from about five PNs on average. We are currently testing the effects of stimulating different numbers of presynaptic neurons and using different stimulation strengths to get an estimate of how many presynaptic neurons must be active and at what spike rate in order to evoke a spiking response in a MB neuron.

Our second approach is to directly examine the inputs to MB neurons, using functional imaging to characterize those inputs where they synapse onto the dendrites of the MB neurons. Activity of these synapses leads to calcium influx, which we detect using synthetic fluorescent calcium indicators. We load these indicators into an individual neuron via a patch-clamp recording electrode. By measuring calcium influx at synaptic sites using two-photon imaging, we can derive an odor tuning curve for individual dendritic inputs while simultaneously monitoring the whole cell's odor response via the recording electrode at the cell body. This approach allows us to directly determine whether different dendritic sites have different odor tuning properties. It also enables us to establish whether multiple dendritic sites must be activated to drive the MB neuron to spike. Ultimately, we hope to generate a model that explains how different dendritic inputs are integrated to give rise to the whole cell's odor-tuning properties.

Neuromodulatory Influences on Olfactory Information Processing

T. Hige, K. Honegger

Neuromodulatory systems such as dopamine have an important role in learning and memory. In *Drosophila,* it is known that dopamine release is both necessary and sufficient for performance in the standard Pavlovian olfactory conditioning assay. The two leading hypotheses for the function of dopaminergic inputs are (1) they convey information about the shock punishment and (2) they control arousal levels and influence the intensity of the animal's sensory responses. At the circuit level, these two hypotheses make two different predictions. The first hypothesis predicts that dopamine influences synaptic plasticity, either increasing the competence for plasticity to occur or prolonging short-term plasticity that accompanies neuronal activity. The second hypothesis suggests that dopamine may control the propagation of sensory information through the olfactory circuit. We are testing these two hypotheses by electrophysiologically characterizing neurons downstream from the MB. We have found that dopamine can block sensory responses in these

downstream neurons. This suggests that dopamine controls signal propagation through the MB, and dopamine release can close the gate of information flow, consistent with the second hypothesis above. We are directly testing this possibility by examining signal propagation within the MB using imaging techniques. This model suggests that the salience of a sensory stimulus can be controlled by regulating signal propagation within the brain, potentially providing a mechanistic cellular underpinning that contributes to the fundamental cognitive process of attention.

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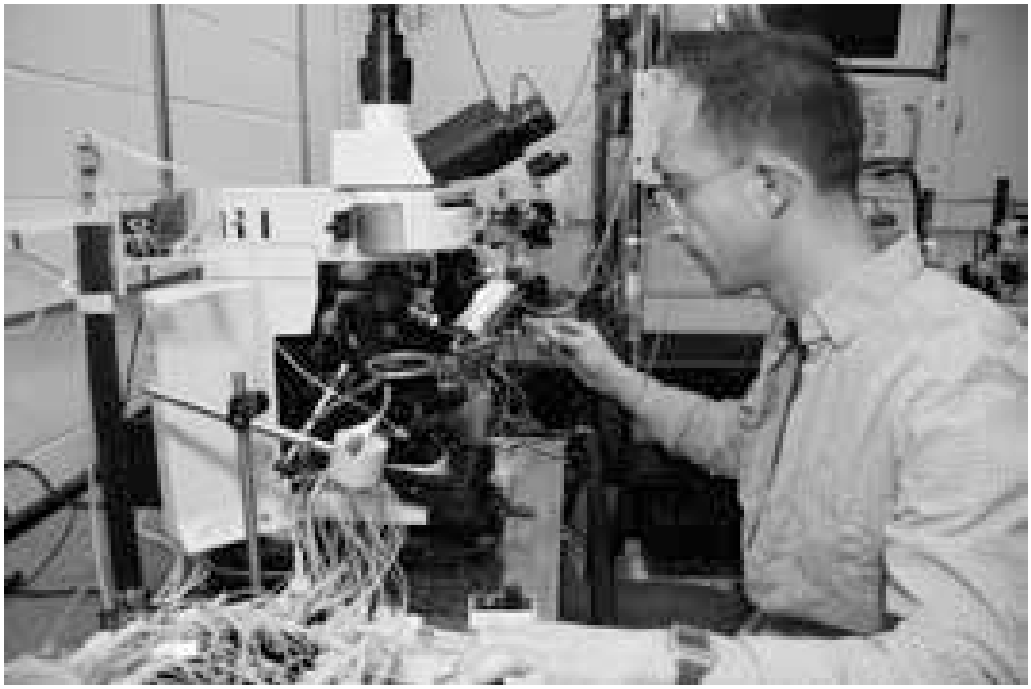

Glenn Turner

NEURAL CIRCUITRY OF UNDERLYING NORMAL AND ABNORMAL PROCESSING IN THE CORTEX

A. Zador K. Borges, T. Hromadka, S. Jaramillo, S. Koh, 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. We are currently using several mouse models of autism, including MeCP2, PTEN, and neuroligin, to test this hypothesis.

At the in vitro level, we are using adeno-associated virus (AAV) to express channelrhodopsin-2 (ChR2, a light-activated cation channel) in the axons of neurons which project to the auditory cortex. We then use whole-cell patch-clamp recording in acute auditory cortex brain slices to compare the light-evoked response properties between wild-type neurons/mice and autistic neurons/mice.

Cholinergic Modulation of Auditory Responses

T. Hromadka [in collaboration with S. Ranade and A. Kepecs, Cold Spring Harbor Laboratory]

The cholinergic neuromodulatory system originating from nucleus basalis has been implicated in many interesting brain functions, such as attention and cortical plasticity. Traditional methods of studying cholinergic modulation of neuronal activity usually either have rather poor spatial resolution, as direct stimulation of nucleus basalis leads to a diffuse modulation of cortical activity in the entire brain, or provide imprecise temporal control in case of direct acetylcholine application into a specific cortical area.

We are using optogenetic techniques with extra/intracellular recordings to study cholinergic modulation of sensory processing in the auditory cortex of mice. Spatiotemporal precision of acetylcholine release is provided by selectively expressing ChR2 in nucleus basalis neurons projecting to auditory cortex. We record sound-evoked responses of single neurons while selectively modulating neuronal activity "on-demand" by shining brief pulses of blue light onto the cortical surface, which selectively releases acetylcholine from axons of cholinergic neurons projecting from the nucleus basalis.

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

G. Otazu, Y. Yang

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 the 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. 2009).

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 to be 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, which combines 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.

Development of a Novel Strategy for Expressing Transgenes in Mice

K. Borges

The expression of transgenes in mice is a standard technique used in many areas of biological research. However, establishing new transgenic lines can be difficult for a number of reasons. For example, expression can be highly dependent on where the transgene is located in the genome.

We have developed a novel approach for transgene expression. Our transgenic constructs comprise a gene of interest followed by a promoter, with flanking *lox* sites. In this configuration, the transgene will not be expressed, as it precedes the promoter. However, one can then introduce Cre recombinase, either with viral expression of Cre or by crossing these mice with a separate Cre line. In the presence of Cre, the transgene-promoter segment will be excised from the chromosome and form a circular, plasmid-like construct. Because the promoter will now be upstream of the transgene, the transgene will be expressed.

Because the transgene is "silent" until it is excised from the chromosome, this strategy avoids problems associated with location-dependent effects on expression. In addition, the fact that expression is Cre-dependent makes this approach flexible for a variety of applications. Temporal and spatial control of transgene expression can be achieved with virally mediated Cre expression. Alternatively, the ability to cross the same transgenic mouse line with any Cre-expressing line will allow for cell-type-specific expression of the transgene.

During the past year, we have designed and made two transgenic constructs, injected them into mouse embryonic stem cells, and identified founder mice. These mice are now being bred, and once we have colonies of sufficient size, we will use a Cre virus to test whether expression can be achieved after excision of the transgene from the genome.

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 increased evoked response to tones immediately preceding the expected moment of appearance of the target when compared

against 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.

PINP: Identifying Neurons with ChR2 during In Vivo Recording

T. Hromadka, S. Koh

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 we call "PINP" (photostimulation-assisted identification of neuronal populations), which allows us to "tag" subpopulations of neurons for identification during in vivo electrophysiological recordings (Lima et al. 2009). The tag is a light-gated ion channel—the algal protein 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.

Retrograde Tagging of Neurons for Electrophysiological Identification and Manipulation of Activity

P. Znamenskiy

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. One attractive hypothesis is that this functional diversity reflects in part the anatomically and molecularly defined neuronal subtypes. Activity of neurons with different patterns of projections also undoubtedly has distinct roles in behavior. To test these hypotheses would require a tool to selectively tag and manipulate populations of neurons based on their projection targets. Expression of ChR2, a light-gated cation pump, can serve as such a tag. ChR2-expressing neurons can be identified in electrophysiological recordings through their responses to light. Light can be used to manipulate their activity with millisecond precision.

Viruses capable of retrograde transport may provide a method for restricting ChR2 expression to an anatomically defined subset of neurons. We have previously used recombinant HSV to tag callosal projection neurons in auditory cortex (Lima et al. 2009). However, this method is apparently limited because it appears to tag mainly intracortical projections. We are therefore developing an alternative to HSV: pseudorabies virus (PrV) amplicons.

PrV amplicons also have the added benefit of extraordinary packaging capacity (up to 150 kb). This opens up the possibility of using large regulatory elements to tag neuronal subtypes defined by expression of molecular markers, akin to BAC (bacterial artificial chromosome) transgenics. However, unlike BAC transgenics, such a method would be applicable in any species. We are exploring the possibility of using PrV amplicons to target specific subtypes of interneurons (such as parvalbumin- and somatostatin-expressing neurons) and neurons producing specific neuromodulators (acetylcholine and dopamine).

Causal Role of Auditory Cortex in Behavior

P. Znamenskiy

An understanding of the causal role of populations of neurons in behavior requires manipulations such as in-

activation or stimulation. To better understand how neurons in the auditory cortex contribute to decisions driven by sounds, we have devised a two-alternative forced-choice auditory discrimination task optimized for conducting microstimulation experiments. In this task, rats are presented with strings of short overlapping pure tones of different frequencies and are required to discriminate whether high-frequency (20–40 kHz) or low-frequency (5–10 kHz) tones were more frequent in the stimulus. Although the basic discrimination (high vs. low) is very easy, the task can be made arbitrarily difficult by decreasing the difference in the frequency of high and low tones. To respond correctly on such difficult trials, rats must integrate sensory evidence over extended stimulus epochs. This task is ideally suited to study the role of the auditory cortex through microstimulation. Because high and low tones are an entire octave apart, their representation within the primary auditory cortex is separated by ~1 mm. Therefore, by stimulating different regions of the auditory cortex it should be possible to "inject" sensory evidence into the circuit, thus biasing the rat's responses. The next step will be to study the contribution of different neuronal subpopulations identified by their molecular markers or anatomical connections to decision making using ChR2-assisted photostimulation.

Mapping of Auditory Cortex Circuitry Using Laser-scanning Photo Stimulation

H. Oviedo [in collaboration with I. Bureau, Cold Spring Harbor Laboratory, and K. Svoboda, HHMI, Janelia Farm Research Campus, Ashburn, Virginia]

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 (LSPS), to map the circuitry within the rodent auditory cortex. Using this approach, we can directly compare the circuitry within the auditory cortex to that of other sensory cortices, such as the better-studied barrel cortex.

Surprisingly, we found that local inputs to cortical layers 2 and 3 differ depending on whether or not the auditory cortex is cut to preserve information across frequencies. Along the anteroposterior frequency axis, input to layer 3 (but not layer 2) arises predominantly out of column, whereas along the dorsoventral isofrequency axis, connections are mostly columnar. Furthermore, this circuit anisotropy is associated with functional differences in sound-evoked responses: Layer-3 neurons receiving out-of-column input are not responsive to simple pure tone stimuli. These results suggest that the microcircuitry of auditory cortex is specialized to the unique one-dimensional representation of sound in the auditory cortex.

Different Sensory Cortical Areas Have Different Sensitivity to Cortical Timing Information

Y. Yang

It is well established that animals can exploit the fine temporal structure of some stimuli; for example, interaural time differences of <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. To address this question directly, 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. To bypass subcortical auditory pathways, we stimulate the cortex directly, using transient biphasic current trains delivered via chronically implanted intracortical microelectrodes. Using this approach, we have previously reported that the auditory cortex can make use of information on a time scale as short as 3 msec. We have now extended this approach to compare auditory cortex with other brain areas.

To determine whether the auditory cortex is unique in its ability to utilize such fine timing differences to drive behavior, or if the cortex operates according to general principles shared across different regions, we compared the ability of different sensory areas to resolve subtle differences in neural timing. In the visual cortex, we found that although animals could be trained to resolve differences as short as 15 msec in neuronal activity, they could not resolve differences as short as 5 msec. This lower limit of 5–15 msec was significantly higher than the limit of 3 msec we observed in the auditory cortex and is consistent with the view that the visual cortex is "slower" than the auditory cortex. Surprisingly, we found that the barrel cortex was even "faster" than

the auditory cortex, with a lower limit below 1 msec. Our results suggest that different cortical areas are differentially able to derive behaviorally relevant information from the fine timing of neural activity.

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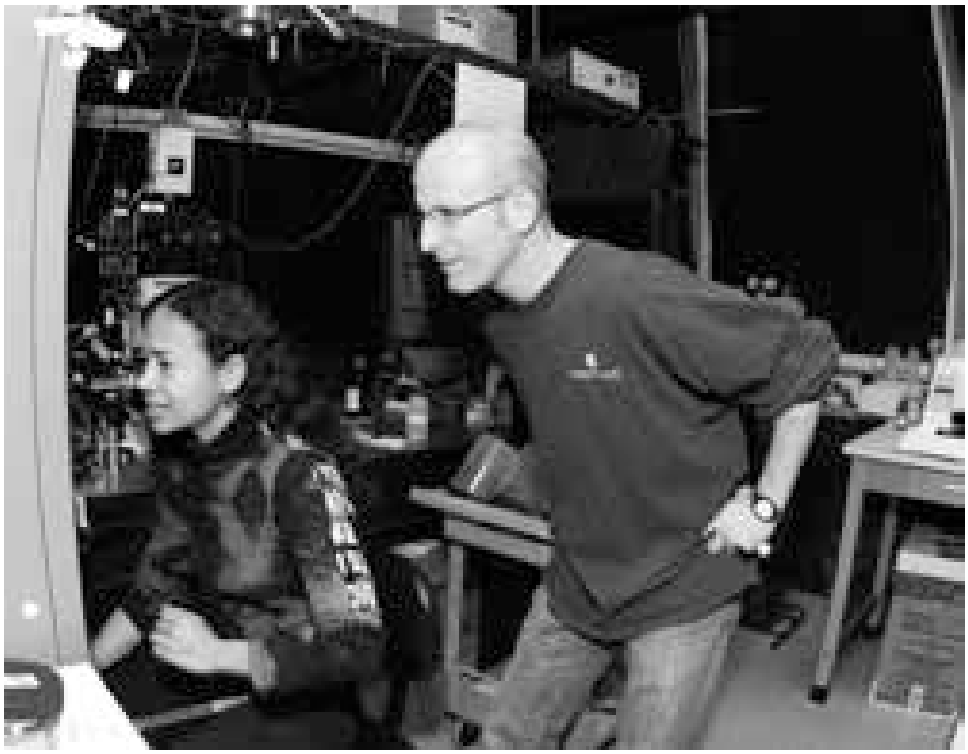

Hysell Oviedo, Tony Zador

NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong J. Beshel L. Wang
R. Pagani C. Xu

Molecular Basis of Long-term Memory Induction

R. Pagani

Memory that is induced by repeated training improves when presentations are spaced over time compared to equal numbers of training exposures without spacing. This augmentation in memory formation is called the spacing effect and is a common phenomenon in the animal kingdom. The spacing effect has been widely studied in both basic and applied research because of its relevance for psychology, education, therapy, and advertising.

In the course of studying *Drosophila* models of Noonan syndrome (NS), we discovered that disease-associated mutations hampered memory formation and altered the spacing effect. NS is an autosomal dominant genetic disorder characterized by facial dysmorphia and other developmental abnormalities, including learning difficulties and mental retardation. Mutations causing NS have been identified in five genes, *PTPN11, SOS1, RAF1, BRAF*, and *KRAS*, all of which encode proteins that are components of the Ras/mitogen-activated protein kinase (MAPK) signaling pathway. Gain-of-function (GOF) germline *PTPN11* mutations cause ~50% of NS cases. The *PTPN11* gene and its ortholog in fruit flies, *corkscrew* (*csw*), encode the evolutionarily conserved protein tyrosine phosphatase SHP2, which is recruited to many receptor tyrosine kinases upon activation and is generally a positive regulator of Ras/MAPK signaling.

Recently, we identified *csw* as a genetic factor controlling the spacing effect in *Drosophila* (Pagani et al. 2009). We found that *csw* gain-of-function mutations impair long-term memory (LTM) formation by prolonging the length of the resting intervals between repetitive training required for induction of LTM. This prolonged resting interval correlated with altered training-induced MAPK activity within each resting interval.

PI3K Signaling Is Involved in Aβ-induced Memory Loss in *Drosophila*

L. Wang

Genetic studies of early-onset familial Alzheimer's disease (AD) provide a causative link between AD and β-amyloid (Aβ) peptides. A body of evidence suggests that Aβ peptides are capable of modifying a number of biochemical pathways, such as phosphoinositide-3 kinase (PI3K), c-Junk, caspase, and cyclin-dependent kinase-5 (CDK-5). An alteration in these pathways may lead to a wide range of cellular dysfunctions, such as disturbed metal ion homeostasis, Ca^{2+} dysregulation, and impaired neurotransmission. However, how Aβ42 alters these signaling pathways and how these alterations lead to pathological phenotypes have not been well studied.

We used a transgenic *Drosophila* AD model to identify the biochemical pathways through which Aβ42 induces age-dependent memory loss, the early hallmark of AD. With electrophysiology and immunohistochemisty studies, we found a hyperactivated PI3K pathway in Aβ42-expressing flies. Our following Pavlovian olfactory conditioning tests indicated an amelioration of immediate memory loss after inhibition of PI3K activity. Furthermore, this reduced PI3K activity correlated with reduced Aβ42 accumulation in fly brains, including both oligomer and fibril aggregates. Because no recovery of neurodegeneration was found in Aβ42-expressing flies with inhibited PI3K, we speculate that the Aβ42-induced memory deficit and neurodegeneration are mediated through different signaling pathways.

State-dependent Odor Processing in the *Drosophila* Mushroom Body

J. Beshel

An animal's internal milieu may have a significant role in facilitating odor perception in addition to developing

and expressing odor memory. The relative simplicity of the *Drosophila* nervous system makes it an attractive candidate in which to investigate how alterations in the internal state affect sensory processing and ultimately behavior. Recent studies have demonstrated that the satiety state has a profound impact on the expression of memory in flies, and genetic dissection has pointed to a subset of neurons innervating the higher-order olfactory center—the mushroom bodies (MBs)—as important for mediating these changes. Yet, there remains no direct evidence how such neuromodulation modifies MB processing to lead to appropriate behavioral expression.

Using two-photon laser-scanning microscopy in combination with the P-Gal4 enhancer trap system to target the expression of the Ca^{2+} probe G-CaMP in specific subsets of MB neurons, we monitored in vivo odor-evoked activity profiles of flies under differing levels of satiety. As predicted by previous work, hungry and fed flies displayed varying responses to monomolecular odorants at the level of the MB vertical lobes. This effect, however, was not limited to MB output. Responses in the calyx, the dendritic region of MB intrinsic neurons, also varied as a function of satiety, suggesting that modulation set in motion by hunger does not simply function to open a "gate" for MB output. Rather, hunger may also serve to shape odor representations at the level of MB input.

Such multiple controls over olfactory system circuitry may allow the animal to navigate its environment, surrounded by vast amounts of odor information, while retaining sensitivity of olfactory function to what is relevant without the expenditure of energy resources on what is not. Future experiments will seek to address whether the changes in MB activity are related only to the expression of acquired memories in flies or whether the satiety state can modify even more basic behaviors such as olfactory acuity or innate odor preferences.

Mechanosensory Integration in *Drosophila*

C. Xu

One of the fundamental questions in neuroscience is how sensory inputs are represented in the central brain. The fruit fly is a well-established model for studying olfaction and olfactory-associated learning and therefore can help us to address such questions. Our previous findings suggest that the MB, the second neuronal relay in the olfactory pathway that is also known as a learning and memory center in the fruit fly, responds to near-field mechanical stimuli, i.e., air flow.

We are able to express the genetically engineered high-sensitivity calcium indicator G-CaMP in most neurons in the MB by using enhancer-trap lines. With two-photon microscopy, we can monitor the activities of MB neurons in vivo. The "snapshots" indicate that in the calyx, the input site of the MB, the turning profile to different flow rates of pure air flow is different from that of air flow carrying odorant; the activity pattern in response to both odor and air flow in the calyx seems not to equal the linear sum of the activity patterns for each. All of this suggests that olfactory and mechanical sensory stimuli interact with each other in the MB, at least at the input level.

Future work will include analysis of the activity profile of MB neuron cell bodies and MB lobes. This may lead to an understanding of how these two senses interact at the output level of the MB, and consequently if, and, or how this affects flies' behavior.

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PLANT GENETICS

Uncovering the molecular processes governing how plants grow and develop is of fundamental biological interest and importance, with vital implications not only for agriculture, but also for human health. *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 in work on maize in the 1940s. Plant geneticists at the Laboratory have been among the leaders of efforts to sequence the first plant genomes, and they continue to be at the center of sequencing and genome-annotation projects involving a host of cereal crops that feed the planet's growing population. CSHL plant geneticists also have been pioneers in the study of RNA interference, in stem cell research, and, most recently, in efforts to spur the development of next-generation biofuels and related alternative energy sources.

David Jackson and colleagues study genes and signals that regulate plant growth and architecture. This past year, they identified a gene called *GAT1* 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. *GAT1*, they discovered, encodes an enzyme found mainly in meristems, the stem-cell-rich tip of the plant where new growth takes place. The enzyme improves the flow of traffic through plasmodesmata by acting as an antioxidant. The lab continues to work on *RAMOSA3*, a gene, first identified by Jackson several years ago, which gives rise to an enzyme that, among other factors, controls branching in inflorescences. Jackson has also undertaken to develop 100 maize lines that express fluorescent proteins across the full spectrum of cellular compartments. These are being made available to the broader scientific community, enabling observations of processes never before seen in living plants. A team that included Jackson, for instance, has drawn upon this resource to identify a gene called *sparse inflorescence1* that proves vital in the formation of corn ears.

Zachary Lippman's research focuses on understanding how tomato plants are elegantly programmed through their DNA code to generate a specified number of flowers in a repetitive zigzag arrangement on a branching structure called an inflorescence. Proceeding from a base of knowledge they have built regarding the special repetitive growth habit represented by tomato, called sympodial growth, Lippman's lab is addressing basic and applied questions on how plants are able to shift from making leaves to making flowers, and the number of flowers different types of species are ultimately able to make. The lab is particularly interested in learning how these vegetative-to-reproductive "phase transitions" were involved in the evolution of diverse inflorescence branching patterns in tomato's larger *Solananaceae* family, which ranges from simple (plants with a single branch carrying a single flower) to complex (plants with dozens of branches harboring dozens of flowers). Their aim is to decipher both the developmental and molecular bases 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 DNA and the chromatin that bundles it—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 his colleagues have shed light on a phenomenon called position-effect variegation, 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, Martienssen and colleagues described a remarkable process by which "companion cells" to sperm in plant pollen grains provide them with instructions that protect sperm DNA from damage. Com-

panion cells lose epigenetic marks in genomic regions containing transposons, resulting in their activation. Short interfering RNAs (siRNAs) bearing the epigenetic information are then transported from the companion to the sperm, effectively inoculating the germline against transposon damage. Martienssen's group also continues to work on problems related to the creation of plant-based biofuels.

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 also used mutational analyses to unravel the mechanism that suppresses stem cell fate during organ development. They have shown that this process requires a highly conserved epigenetic mechanism. In particular, they have found that the chromatin remodeling factor HIRA, through interaction with specific DNA-binding proteins, mediates the recruitment of polycomb repressive complexes to stem cell factors, an action that stably represses their expression in differentiating organs. The lab has also identified regulatory mechanisms that allow for the precise spatial accumulation of developmentally important small RNAs in plants. This work has revealed that polarity in leaves is established via opposing gradients of mobile small RNAs. 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, P. Borrill, K. Chen, S. Choi, S. DeBlasio, A. Eveland, S. Goldshmidt, D. Heigl, U. Hernandez, R. Johnston, G. Nguyen, Y.K. Lee, L. Panda, M. Pautler, J. Wang, C. Whipple, 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 each other through small channels, called plasmodesmata. These channels were described in plants more than 100 years ago, but their central importance and how they are regulated is 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 controlled via regulation of protein folding, by a chaperonin gene that regulates the specific transport of a transcription factor, KNOTTED1. KNOTTED1 is a homeodomain protein that is expressed in the 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, *compact plant2*, encodes a subunit of a heterotrimeric G protein that has previously not been linked with stem cell proliferation in plants. 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 can be used in crop improvement.

A Role for Chaperonins during KNOTTED1 Cell-to-Cell Trafficking

X.M. Xu, J. Wang, P. Borrill, L. Panda

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 such as transcription factors through plasmodesmata (PDs), channels that traverse the cell wall and connect plant cells to their neighbors. In addition to cell-fate specification, PDs also function 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, we are taking an unbiased genetic strategy to dissect these molecular components and mechanisms, using a transgenic reporter 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 *glabrous1* (*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, a mutant with attenuated KN1 trafficking was identified as encoding a chaperonin. Using artifical microRNA (miRNA) knockdowns, we found that additional chaperonin sub-

units also function during KN1 trafficking, suggesting that the whole chaperonin complex is required for trafficking. Furthermore, genetic interaction data provide evidence that the endogenous function of KN1-related genes in stem cell maintenance in *Arabidopsis* requires chaperonin activity, clearly supporting the functional relevance of chaperonin-mediated trafficking through PD.

The central question is how a chaperonin protein might be involved mechanistically 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. Meanwhile, it is also possible that chaperonins may function during posttranslocation 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. Our preliminary analysis through cell-type-specific complementation experiments strongly suggests that chaperonins are involved during posttranslocational refolding. In summary, a functional characterization of chaperonins, the first factor known to be critical for KN1 PD trafficking, has furthered our understanding of developmental regulation and mechanisms of selective cell-to-cell trafficking.

Generation of Fluorescent-protein-tagged Maize Lines for High-throughput Analysis of Protein Localization and Function

S. DeBlasio, T. Zadrozny, A. Goldschmidt, C. Whipple, U. Hernandez, J. Wang [in collaboration with A. Chan and Y. Xiao, The J. Craig Venter Institute, and A. Sylvester, University of Wyoming]

With the recent release of the maize B73 genome sequence, we have entered into a new scientific era where computational genome annotations must now be validated or deduced by experimentation in order to truly understand the precise function(s) of all maize proteins as well as identify the regulatory elements controlling their expression. Even though annotation algorithms are based on published experimental data such as homology with genes/proteins that have previously been characterized, the functional information assigned to maize gene sequences can only be considered a prediction. In fact, there are still many cases where a putative function/localization could not be assigned to a sequence due to lack of homology with orthologous sequences. In addition, most current sequence analysis programs are unable to identify proteins that might be targeted to more than one cellular compartment or might have more than one function. Although there are several approaches to experimentally validate gene function, none are more commonly used than fluorescent protein (FP) technology.

Successfully optimized for use within living plant cells, FPs genetically linked to a protein or regulatory element of interest make the normally invisible protein of interest visible, and easy to track. Because of their relatively small size, FP fusions rarely interfere with native protein targeting, function, and trafficking. Thus, FP technology is a powerful tool that can be used to noninvasively mark protein expression and localization to study various subcellular processes, live and in vivo.

Maize cells consist of interconnected but discrete compartments that help to maintain cellular function and order. Because identifying proteins that localize to these domains is critical to fully understanding maize physiology and development, we have set out to generate 100 stable, natively expressed, FP fusion lines to provide a useful, molecular resource for the maize community and to ultimately uncover guiding information for crop improvement. From the lines developed thus far, we now have unprecedented views of maize cellular architecture and can study real-time dynamics of cell structure, function, and protein localization, including known proteins from several hormone signaling pathways, such as the jasmonic acid metabolic enzyme, LIPOXYGENASE10, which shows a distinct, developmental localization pattern in maize inflorescences. The tagged lines provide complete marker coverage of almost all subcellular compartments, including motile organelles such as peroxisomes and nuclear markers such as histone H1, which displays a punctate localization pattern. Where possible, these FP fusion lines have been shown to complement their respective mutant alleles, indicating functional activity. Data on the characterization of these lines, including confocal micrographs, are freely accessible on our public website, http://maize.jcvi.org/cellgenomics/index.shtml. The website also includes a community submission form to request one's favorite gene to be tagged. Constructs and seeds from verified transgenic plants are also available for use by the scientific community. The ultimate goal for the creation of these FP-tagged transgenic plant collections is to provide a molecular resource for the maize community to use for functional studies, such as FRAP (fluorescence recovery after photobleaching), FRET (fluorescence resonance energy transfer), mass spectrometry, and ChIP (chromatin immunoprecipitation).

We are currently working to optimize the LhG4 two-component *trans*-activation expression system, which has been successfully used in *Arabidopsis*, for use in maize. This system uses the transcription factor LhG4, expressed by a cell or tissue-specific promoter, to drive the expression of any reporter in *trans*, via pOp transcription-factor-binding sites. The reporter can be any gene expressed in a tissue-specific manner, such as a fluorescent protein or an FP fusion protein, a gene of interest for tissue-specific expression, a silencing construct for tissue-specific knockdowns, or even a gene encoding a toxin for ablation experiments. Our goal is to generate ~50 promoter constructs and produce an array of cell- and tissue-specific driver lines that we will use to analyze the changes which occur within meristems during differentiation and development. From our previous work creating the natively expressed FP fusion lines to highlight subcellular compartments, we have already identified several tissue-specific promoters, including *a-ZEIN* (endosperm) and *ZmMADS16* (floral organ primordia) (Fig. 1).

Digital Expression Signatures during Maize Development

A.L. Eveland, N. Satoh-Nagasawa, M. Regulski, A. Goldshmidt [in collaboration with D. Ware, Cold Spring Harbor Laboratory; M. Beatty and H. Sakai, DuPont Crop Genetics; R. Schmidt, University of California, San Diego; and E. Vollbrecht, Iowa State University]

Genome-wide expression signatures that mark perturbations in developmental programs contribute to identification and functional analysis of central regulators and associated pathways. In this work, we are using next-generation sequencing to profile genome-wide transcriptional changes in maize reproductive tissues at specific stages of development. We also use mutations in key developmental genes to test the effect of gene-specific perturbations in normal reproductive growth at the transcriptional level. Genetic studies have previously anchored these genes into core developmental pathways. Here, computational methods are used to integrate and analyze expression data sets, which will provide a basis for inferring the gene networks associated with these pathways.

A primary objective of this work is to resolve transcriptional mechanisms that modulate branch patterning in maize inflorescences. Branching architecture is an important agricultural trait with clear relevance to grain yield and harvesting ability. In maize, male and female flowers develop in separate inflorescences called tassels and ears, respectively. In both structures, branching patterns are determined by developmental fate of a series of axillary meristems (highly organized stem cell populations), which are initiated during early development. Loss-of-function mutations for genes in the *RAMOSA* (*RA*) pathway have demonstrated a common effect on determinacy of axillary meristems and thus result in highly branched phenotypes in the ear and tassel. To investigate additional regulatory and biochemical processes that contribute to meristem determinacy, we are using Illumina deep-sequencing strategies to compare genome-wide transcript profiles from *ra1*, *ra2*, and *ra3* mutant ears during different developmental stages. Expression profiles from normal ears and tassels are being analyzed in parallel, and these include samples enriched for meristematic cells taken from sections along the developing inflorescences. The Illumina-based sequencing methods currently being used include tag-based digital gene expression (DGE) and RNA-Seq.

An initial data set, which compared transcript profiles of immature (2 mm) *ramosa3* mutant ears to wild-type using DGE, was used to generate a framework for analysis of tag-based expression data in maize. The *RA3* gene encodes a trehalose-phosphate phosphatase (TPP), and determining its function could reveal a link between developmental and metabolic processes during maize inflorescence development. From these data, we identified ~23K high-confidence genes expressed in immature ears and used comparative genomics to leverage

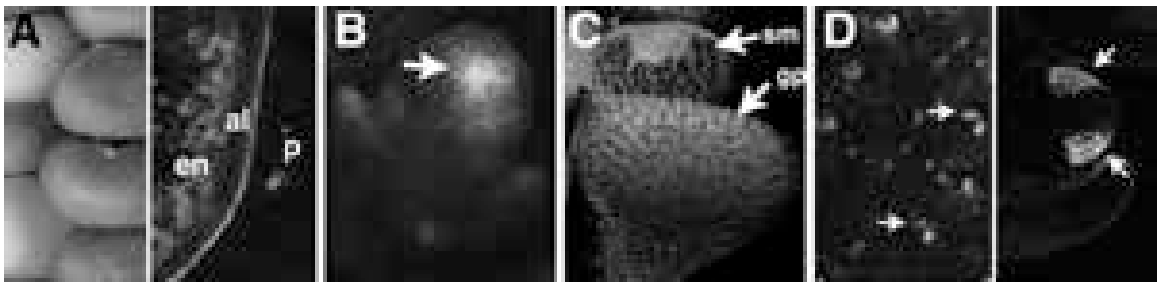

Figure 1. Tissue-specific fluorescent maize reporter lines. The images are confocal micrographs showing expression of fluorescent protein-tagged proteins in endosperm (*A*), meristem stem cells (*B*), glume leaf primordia (gp) (*C*), and floral promordia (*D*). Promoters discovered from this work will be used to create a *trans*-activation expression resource.

information from *Arabidopsis* and rice in functional analyses of differentially expressed maize genes. These included key enzymes in relevant biochemical pathways and regulatory components such as transcription factors and miRNAs. Candidate genes from these results have been identified and will be used in downstream genetic and molecular analyses to further characterize their role with respect to *RA3* and control of branching.

Currently, we are constructing and sequencing DGE and RNA-Seq libraries from normal and mutant maize ears. A pipeline for mapping and analysis of RNA-seq data with the newly assembled maize reference genome is being optimized in collaboration with Doreen Ware's lab. The sequence-based expression data sets will then be integrated to generate a transcriptional framework, which can be continually referenced and improved as new data sets become available. Including genome-wide analyses of single loss-of-function mutants in this framework enables us to classify transcriptional changes that result from specific perturbations of the system. Incorporation of additional data from ChIP-Seq and yeast–2-hybrid experiments (currently under way) will provide genome localization and protein–protein interaction information for the *RA* genes as well as other candidates, and thus a more comprehensive view of molecular mechanisms underlying branching architecture in maize inflorescences.

Regulation of Meristem Size in Maize and Its Impact on the Evolution of Inflorescence Morphology

P. Bommert, Y.K. Lee, M. Pautler, D. Heigl, S. Choi [in collaboration with M. Komatsu and H. Sakai, DuPont Crop Genetics]

One of the most remarkable morphological changes observed during the domestication of inflorescences in maize is the change from a two-rowed ear in teosinte, the ancestral form of maize, toward the multirowed ears of modern elite maize hybrid lines, which have up to 24 rows of seeds. We found that this change in seed row number is tightly linked to the size of the inflorescence meristem (IM), which is constituted by a group of stem cells located at the tip of developing ears. It is also known that this change in morphology is dependent on the activity of multiple genes. Our goal is to identify and analyze these genes controlling the size of the inflorescence meristem and thereby controlling the important agronomical trait of seed row number. Maize is a good system to identify these genes, because the ear appears to be very sensitive to changes in gene activity, most likely as a result of domestication, and ear and tassel meristem phenotypes are easy to identify even in case of weak mutations.

One of these mutations is *compact plant2 (ct2)*. *ct2* mutants are significantly shorter than their wild-type siblings, but more interestingly, *ct2* mutants are characterized by enlarged IMs and vegetative meristems, resulting in severely fasciated ears and in an increased tassel spikelet density. We used a map-based cloning approach to isolate *ct2.* Using ~900 F_2 mutants, we narrowed down the chromosomal location to an interval of ~250 kb on chromosome 1, which is highly syntenic to rice and sorghum. By sequencing several candidate genes in the different *ct2* alleles, we found that *ct2* encodes the α subunit of a heterotrimeric G protein. Heterotrimeric G proteins form signal transduction complexes composed of three dissimilar subunits: Gα, Gβ, and Gγ. In *Arabidopsis*, maize, and rice, Gα is encoded by a single-copy gene and is involved in multiple aspects of growth and development, including cell proliferation and cell elongation. However, a link to stem cell proliferation has not been previously established. Initial analysis of transgenic plants expressing CT2 fused to yellow fluorescent protein (YFP) indicates that CT2 protein is strongly expressed within meristems. This will allow not only tissue and subcellular expression analysis, but also proteomic applications using YFP as a purification tag. Previous studies have linked rice Gα with giberellic acid hormone signaling, and we found that the GA synthesis rate-limiting enzyme GA20 oxidase is significantly down-regulated in *ct2* mutants, suggesting a novel link between GA action and meristem size regulation.

Another gene that we are in process of cloning using a map-based cloning approach is *fasciated ear3 (fea3*). *fea3* mutants are affected during female and male inflorescence development, as they develop fasciated ears with an extra number of kernel rows and tassels with an increased spikelet density. Using a total of 950 recombinants, we narrowed down the *fea3* region to ~800 kb on chromosome 3, which is covered by five bacterial artificial chromosomes (BACs) representing 15 genes. In addition to the *fea3-Reference* allele, which is of unknown origin, we successfully isolated additional *fea3* alleles by a targeted ethylmethanesulfonate (EMS) screen as well as by bulked segregant mapping of other fasciated mutants. The 15 candidate genes are currently being sequenced in these *fea3* alleles to identify the *FEA3* gene.

We are also mapping a newly identified mutant, *fea 1905,* that has a thickened tassel and massively fasciated ears due to enlarged IMs. We have mapped the mutation to a small interval on chromosome 6, containing ~80 annotated genes, and continue to narrow down its position and sequence candidate genes. We have crossed *fea 1905* to *ra1* and *ra3*, as well as other fasciated ear

mutants to identify further genetic interactions. Future work includes phenotypic characterization, as well as expression analysis, to elucidate function and mode of action for these novel genes. We are also mapping a collection of ~40 additional fasciated mutants by bulked segregant analysis using the Sequenom platform. We expect some of them to be allelic to each other or to some of the already cloned or mapped fasciated mutants. However, we also expect some to represent new genes involved in IM size regulation.

We also continue to study the *fasciated ear2 (fea2)* gene, which was cloned in our lab several years ago. *fea2* encodes an extracellular receptor involved in stem cell homeostasis. *fea2* mutants are characterized by "fasciated" or enlarged ears, which have more than 40 rows of seed. We found that *fea2* maps to a major quantitative trait locus (QTL) controlling seed row number and are currently analyzing *fea2* expression levels within the IM in the parental lines of the QTL population; we expect to find a correlation between *fea2* expression level and IM size. Besides this molecular approach, we also searched for hypomorphic alleles to further substantiate our hypothesis that allelic variation of *fea2* contributes to variation in seed row number. Using the maize TILLING resource, we isolated four new EMS-induced *fea2* alleles, of which one of them has a single amino acid change within the extracellular receptor domain. Interestingly, homozygous mutants of this line show a significant increase in row number (from ~16 to ~18) without disturbing the overall ear morphology, indicating the potential of the *fea2* gene to alter IM size and seed row number within a range similar to that found in natural populations.

Investigation of Molecular Mechanisms Controlling Meristem Determinacy and Inflorescence Branching in Maize

S. DeBlasio, M. Pautler

We are interested in studying the development of the 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 the 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* acts 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–2-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. The 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, like some metabolic enzymes in yeast (e.g., hexokinase), may have an additional role in transcriptional regulation.

To test this hypothesis, we are currently verifying RA3 interacting proteins in planta using coimmunoprecipitation experiments in a transient tobacco leaf expression system using epitope tagged proteins. Further characterization of the putative interactors, including expression analysis via in situ hybridization, is currently in progress. We have also obtained transgenic plants harboring a fluorescently tagged ZF-HD protein identified from the Y2H screen. The targeted approach described above will be complemented by immunoprecipitation coupled with mass spectroscopy conducted using extracts from transgenic maize plants. We now have green fluorescent protein (GFP) and FLAG-tagged RA3 transgenic plants available, and we are screening for strongly expressing stable lines. We will also use these lines to directly assay whether RA3 occupies the promoter of *RA1* in vivo, using ChIP, and search for other possible binding sites in the genome using ChIP-Seq.

In summary, these diverse approaches will elucidate the mechanisms of inflorescence stem cell determinacy and branching, providing candidate genes for crop improvement.

Characterization of Mechanisms Regulating Spikelet-pair Meristem Determinacy

S. Goldshmidt, M. Pautler

We are using the maize mutants, *ramosa1* (*ra1*), *ramosa2* (*ra2*), and *ramosa3* (*ra3*), which show an indeterminate phenotype, to characterize molecular networks regulated by these genes. Several approaches are being applied for

this purpose. First, we use Illumina transcriptome analysis to identify genes regulated by RAMOSA gene activity. We first performed detailed analysis of ear phenotypes in *ramosa* mutants. We found that elongated branches in ears of *ramosa* mutants could be detected in ear primordia larger then 1.5 mm, when the first spikelet meristems are detected in wild-type ears. On the basis of this analysis, we collected two sets of ears (1–1.5 mm and 1.5–2 mm) for every mutant and wild-type genotype (Fig. 2). RNA is currently being used for construction of Illumina RNA-Seq libraries for whole transcriptome analysis before and at the very early stage after manifestation of the phenotype. This way, we will be able to compare relative changes in transcriptome profiles of the mutant and wild-type plants, before mutant phenotypes are obvious, as well as dynamic changes in the transcriptome profiles during transition of the spikelet-pair meristem into the spikelet meristem stage.

We are also taking a genetic approach to identify genes participating in the *RAMOSA* network. We performed an EMS-based *ramosa3* enhancer/suppressor screen and analysis of double-mutant combinations of the *ramosa* genes with maize meristem maintenance genes, such as *knotted1* (*kn1*), *fasciated ear2* (*fea2*), and *thick tassel dwarf1* (*td1*), and with genes involved in auxin hormone transport and synthesis, such as *barren inflorescence2* (*bif2*) and *sparse inflorescence1* (*spi1)*. Currently, we have identified and verified three unknown suppressors and two enhancers of the *ramosa3* phenotype, and molecular mapping of the genes is under way. We also detected suppression of the *ramosa3* phenotype by a *kn1-e1* loss-of-function mutant; this result supports the previous proposition that *RAMOSA3* has a non-cell-autonomous activity on the maintenance of the spikelet-pair meristem. As an independent genetic approach, we are also mapping a naturally occurring genetic suppressor of *ra3*, discovered when we crossed the mutant to the A619 inbred line. Finally, we are exploiting natural variation in maize by crossing *ra3* to diverse inbred lines that represent the founders of the maize nested association mapping (NAM) population. Initial screening of F_2 families has revealed a strong enhancer of *ra3*, which we will map.

As an additional strategy, in collaboration with Dr. Florin Albeanu at CSHL, we have developed novel two-photon microscopy-based approaches for the imaging of fluorescent-protein-labeled molecular markers. This approach enables us to perform detailed and unbiased analysis of auxin activity markers that report different morphogenetic events during development. Combining these approaches will enable us to better characterize the mechanisms of RAMOSA activity, and the activity of genes that participate in the RAMOSA network.

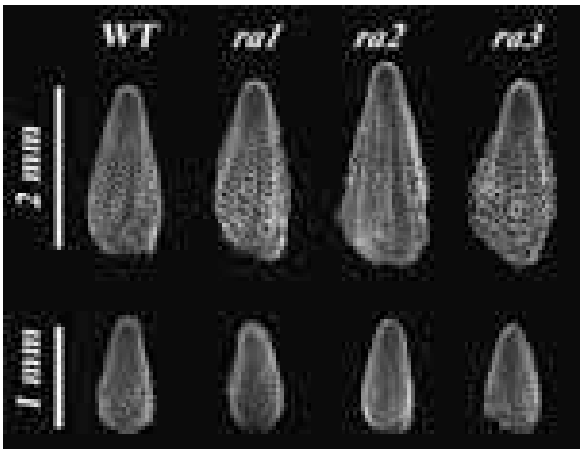

Figure 2. Developmental stages of maize ear primordia used for RNA-seq expression profiling.

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PLANT DEVELOPMENTAL BIOLOGY, STEM CELLS, AND FLORAL BRANCHING SYSTEMS

Z. Lippman K. Liberatore
C. MacAlister
S.-J. Park

One of the most remarkable manifestations of plant evolution is the diversity of floral branching systems called inflorescences. The number and pattern of branches within inflorescences are determined by specialized groups of pluripotent stem cells at growing tips called shoot apical meristems (SAMs). In contrast to the near complete consumption of stem cell populations for organ formation during animal development, the stem cells residing in the SAMs of plants integrate environmental signals with genetic pathways to continuously generate new organs and drive reproductive "phase transitions" throughout life. Beyond genetic frameworks, little is known about the molecular networks controlling reproductive phase transitions, especially with respect to distinct growth habits from diverse species. Research in our lab aims to discover the genes, pathways, and networks that control phase transitions in a widely deployed, but poorly understood, growth habit called "sympodial" that is characteristic of most perennial species, including trees. We are taking advantage of developmental, genetic, and molecular tools in the tomato (*Solanum lycopersicum*) to understand how meristems reiterate to generate a multibranched, multiflowered inflorescence. Importantly, tomato is a member of the nightshade (Solanaceae) family that includes other well-known crop plants, such at potato, pepper, eggplant, petunia, and tobacco. Therefore, we anticipate that our discoveries will address questions that are relevant to evolution as well as agriculture.

A related focus in the laboratory is exploring why intercrossing different inbred plants often creates hybrids that produce more flowers, fruits, and seeds than their parents. This increased vigor in hybrids, known as heterosis, is a widespread phenomenon in nature and is a major contributor to food production worldwide. In addition to studying heterosis quantitative trait loci (QTL) derived from wild tomato species that regulate the rate of inflorescence production, we have recently discovered that mutations in flowering genes can cause heterosis. We are now exploring the role of pleiotropic dosage effects of these genes on plant development as a potential explanation for this type of heterosis.

Two Major Genes Controlling Inflorescence Meristem Determinacy and Branching in Tomato

Z. Lippman

Tomato generates a few-flowered inflorescence organized in a zigzag pattern, but there are three classical mutants called *compound inflorescence* (*s*), *anantha* (*an*), and *falsiflora* (*fa*) that bear highly branched inflorescences (Fig. 1). Interestingly, the *s* mutant, which is the only known inflorescence branching mutant in the eudicot lineage that makes normal fertile flowers, resembles many wild Solanaceae species such as *Solanum crispum* that have highly branched inflorescences. One major project in our lab is exploring whether similarities between mutant and wild phenotypes suggest that branching complexity in nature is based on the regulation of genes such as *AN* or *S* or the pathways in which they reside. Thus, we are focusing our efforts on understanding the functions of *AN* and *S*, which we have found to encode an F-box protein ortholog of a gene called *UNUSUAL FLORAL ORGANS* (*UFO*) that controls the identity of floral organs (petals, sepals, and so on), and a homeodomain transcription factor related to a gene called *WUSCHEL HOMEOBOX 9* (*WOX9*) that is involved in patterning the embryo within the plant seed. Interestingly, these genes have little or no effect on branching in inflorescences that grow continuously (so-called "indeterminate" monopodial shoots), as is found in *Arabidopsis*. However, we have found that transient sequential expression of *S* followed by *AN* promotes branch termination and flower formation in sympodial plants. The phenotypes caused by mutations in the *AN* and *S* genes indicate that any delay (or reversion) in the formation of a flower from an inflorescence meristem allows for an extended developmental period for branch-

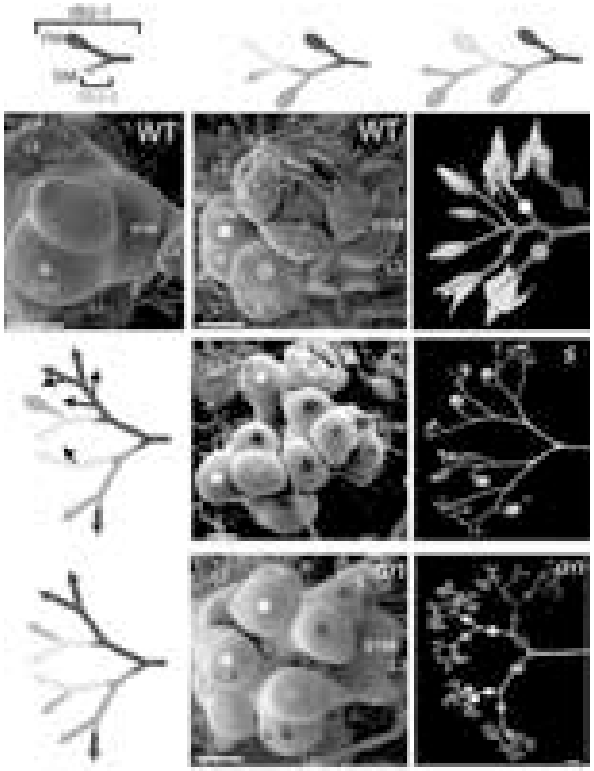

Figure 1. Early branching patterns of normal and mutant tomato inflorescences. Scanning electron micrographs and schematics of inflorescence development. Schematics reflect sequential inflorescence sympodial units (ISUs) each composed of a sympodial inflorescence meristem (SIM) branch (line with arrow) that terminates with a flower (FM, oval). Circles in micrographs reflect corresponding structures in schematics. (*Top panels*) Two stages of sympodial inflorescence development and mature zigzag inflorescence in a normal tomato. (*Middle panels*) *s* inflorescences develop extra SIMs due to mutations in the ortholog of WOX9. Additional SIMs (circles) eventually form flowers. (Black asterisks) Asymmetrical development of meristem branches (black arrows in schematics). (*Lower panels*) Strong alleles of *an*, defective in the tomato ortholog of *UFO*, produce extra SIMs instead of flowers. (L) Leaf; (SYM) sympodial shoot meristem. Bars, 100 m.

ing to occur. In support of the relevance of the *S* gene in nature, we have found that more than 40 domesticated varieties of tomato with branched inflorescences carry mutations in *S.* Given that these plants produce more flowers and fruits, some of these mutant alleles were probably selected by breeders when the tomato was first brought into cultivation more than 200 years ago.

Arguably, some of the most extensive variation in inflorescence complexity is found in the Solanaceae family. For example, the simplest Solanaceae inflorescence is a solitary flower represented by pepper (*Capsicum annuum*), and our research suggests that greater complexity arises from the production of reiterating modular units of stem cells called sympodial inflorescence meristems (SIMs). For example, in tomato, an additional lateral SIM forms before formation of each flower, and this process reiterates to form 6–15 flowers, depending on the variety. In pepper, in contrast, the transition from SIM to flower is very rapid and prevents the formation of additional SIMs. Still, pepper maintains the ability to produce a compound inflorescence from only a single flower, because we have found that mutations in the pepper ortholog of *AN* generate a highly branched structure. Taken together, our findings suggest the existence of a previously uncharacterized developmental mechanism whereby inflorescence elaboration can be controlled through temporal regulation of floral fate.

A Comparative Molecular Study of Inflorescence Development

K. Liberatore, S.-J. Park

Despite growing knowledge on the mechanisms of flowering in some model species (e.g., *Arabidopsis*), it is unclear to what extent vegetative to inflorescence and inflorescence to flower transitions are similar or different in plants such as *Arabidopsis* and tomato. In contrast to the single reproductive phase transition that takes place in *Arabidopsis*, the sympodial habit of the tomato develops three distinct shoot systems, each of which undergoes an independent transition with both morphological and molecular signatures. One project under way in the lab is to study in greater detail the molecular mechanisms that distinguish sympodial from monopodial growth by determining if key branching genes in the tomato are functionally conserved in *Arabidopsis*. We are using a series of transgenic knockdown tools to determine if the ortholog of *S* in *Arabidopsis*, *WOX9*, has a role in inflorescence development and complexity in monopodial species. Curiously, mutations in *Arabidopsis WOX9* have been characterized and have so far been limited to developmental defects restricted to the early stages of embryonic patterning. This early embryonic defect and resulting seedling lethality have prevented a more detailed analysis of later stages of development. By selectively targeting the elimination of *WOX9* using a library of tissue-specific promoters, for example, through RNA interference (RNAi), we hope to determine if and when *WOX9* functions to regulate inflorescence meristem elaboration.

A Comparative Morphological Study of Inflorescence Development

K. Liberatore

How inflorescence meristems develop and branch is only beginning to be studied in diverse species, and there is scarce data for sympodial species. The *s* and *an* mutants are ideal genotypes to serve as genetic and morphological foundations to study the mechanisms that control sympodial inflorescence branching. In particular, *s* is responsible for the majority of inflorescence complexity among domesticated tomatoes, and all strong branching lines found thus far are due to a single allele of *s* (Lippman et al., *PLoS Biol 6:* e288 [2008]). To establish a morphological foundation of how sympodial inflorescence complexity arises during the earliest stages of SIM development, we are carrying out a comprehensive comparative study among Solanaceous plants with different levels of inflorescence complexity. We are using scanning electron microscopy (SEM), in situ hybridization, and transgenic gene knockdown to decipher the "rules" of branching for sympodial species. We are aiming to achieve, for the first time, a detailed temporal compilation of precisely staged meristems representing all points of the vegetative to reproductive transition on the primary shoot and subsequent SIM reiteration.

By comparing *s* and *an* tomato mutants with wild tomato species and other Solanaceae species, we are already establishing a developmental baseline on which to draw conclusions about how SIMs are initiated and reiterated before terminating in flowers. We have screened more than 150 Solanaceae and quantified inflorescence branching, and from these data, we have selected a subset of species that represent a range of architecture types, including *Nicotiana benthamiana*, *C. annuum* (pepper) and *Petunia nyctaginiflora* (petunia) (single flower inflorescences), *Solanum cleistogamum* (two flowers per inflorescense), *Solanum conglobatum* and *Solanum americanum* (tomato-like inflorescence), and *Styrax peruvianum* and *Nicotiana tobaccum* (branched, many flowers per inflorescence). As our model predicts, our preliminary results suggest that the initial SIM of branched species take slightly longer to reach the floral transition, thereby allowing more SIMs to develop.

To deepen our understanding of molecular hallmarks of this transition, we are using comparative in situ hybridization with orthologs of inflorescence meristem and floral meristem identity genes such as *S/WOX9, FALSIFLORA/LEAFY (FA/LFY)* and *ANANTHA/UNUSUAL FLORAL ORGANS (AN/UFO)*. Using these molecular markers, we believe that key steps in the initiation to flowering can be precisely marked, such as boundaries between the shoot apical meristem, the sympodial inflorescence meristem, and floral meristem, perhaps even before a morphological change is seen. In addition, we are using genes such as *FILAMENTOUS FLOWER* and *ANTEGUMENTA* to mark sites of organ initiation, which may subtend each reiterating SIM. These studies will augment the SEM analysis by answering the following questions: Which genes mark the initiation and therefore reiteration of the SIM? Does the existence of a subtending bract (morphologically visible or not) explain the formation of more complex inflorescence types, which are then lacking in single flower species?

The Molecular Dynamics Underlying Altered Developmental Fates of Apical Plant Meristems

S.-J. Park

Mechanisms controlling SAM growth and floral induction have been identified in *Arabidopsis*, as have floral induction genes; however, the dynamic molecular changes that occur during phase transitions have proven to be difficult to study due to physical restrictions. Furthermore, the dynamics of the transition in *Arabidopsis* are not necessarily extendable to other species. We are taking advantage of the three types of meristems of tomato to elucidate the dynamic changes in gene expression that take place during reproductive transitions in plants. Tomato meristems are easily dissected under a stereomicroscope and can be collected in sufficient amounts for RNA extraction. We are growing wild-type plants and precisely harvesting meristems with only two visible leaf primordia every 3 days until floral termination. After the transition, we are collecting SYM, SIM, and FM tissue. This experimental design has provided us with material for expression analyses on eight stages of sympodial growth, with a focus on the temporal dynamics of the vegetative to inflorescence phase transition of the primary shoot. Our preliminary results using marker genes such as *AN, S*, *AP1*, and other floral and vegetative markers suggest that reproductive transitions are not sudden, but rather highly quantitative and dynamic. Furthermore, each meristem seems to have its own molecular signature.

We are now leveraging our microharvesting technique to profile gene expression genome-wide using new sequencing technologies such as Illumina/Solexa (mRNA-Seq) that enable quantitative transcriptome analyses.

Using these data, phase transition markers will be determined based on subsets of genes that show informative expression during SAM termination. Coexpressed genes will be used to identify pathway candidates and promoter motifs, especially as they relate to known developmental and hormone regulatory genes. These profiles will be compared to SYM and SIM transcriptomes to determine the extent to which these phase transitions are similar or different from the primary shoot and each other. This analysis may shed light on a mechanism that explains how SYMs and SIMs are released from dormancy—a process that may be regulated by auxin and other unknown factors. These data will also provide a list of candidate genes for functional studies using reverse genetic tools. Finally, known and newly identified informative genes will be used to create a library of stage-specific promoters to drive fluorescent markers, along with hormone reporters, to visualize expression, first statically and then dynamically in real time. Although the focus of this proposal is on the tomato, the knowledge gained will extend to related Solanaceae and will augment and complement work in nonsympodial plants, such as *Arabidopsis* and grasses.

Novel Inflorescence Branching Mutants

C. MacAlister

To increase our understanding of the pathways and networks regulating inflorescence patterning, we are investigating mutants with novel inflorescence architecture defects. In particular, we are characterizing and cloning the gene responsible for a new tomato mutant called *fasciated flower* (*faf*), which produces a highly branched inflorescence with greatly enlarged flowers and fruits that have increased organ number. This phenotype suggests that some genes have dual roles in regulating determinacy, affecting both the inflorescence and floral meristems. In contrast, *s* and *an* seem to be specific to the inflorescence meristem. Indeed, our analysis of *faf:s* double mutants indicates that the two mutant phenotypes are additive, indicating that these genes operate in separate genetic pathways. Consistent with this, our early SEM data suggest that *faf* mutants begin to manifest an enlarged meristem even in the vegetative phase of development and that this meristem continues to enlarge as the floral phase transition ensues. This results in the formation of multiple ectopic sympodial shoots (SYMs) on the sides of the enlarged primary meristem, which is poised to become an extremely large flower that resembles a sunflower head. Branching also occurs as the SYMs transition to flowering, but to a lesser degree than the primary flowering event. This distinction between the primary and secondary shoot meristems is in line with our preliminary molecular studies which show that these meristems have distinct identities reflected in different molecular signatures. We have already fine-mapped the *faf* mutation, and we have now embarked on gene cloning by sequencing the mRNA from four alleles of *faf* in a common genetic background. If successful, this would be the first example of gene cloning using next-generation sequencing technology in a plant genome as complex as the tomato.

We are also studying a classical mutation called *terminating flower* (*tmf*), which uniquely affects only the primary inflorescence meristem by creating a single terminal flower, whereas all other inflorescences derived from axillary or sympodial shoots develop normally. Interestingly, this mutation was originally reported as being derived from a single tomato plant grafted with a shoot from an eggplant. A somatic mutation ensued resulting in a mutant sector of the plant. Our preliminary work suggests *tmf* has a complex genetic basis, involving at least two unlinked mutations. We are now taking advantage of our recent efforts to sequence a polymorphic wild species of tomato, *Solanum pimpinellifolium*, to generate large mapping populations to identify the responsible genes.

Identification and Characterization of Single-gene Mutations Causing Heterosis in Tomato

Z. Lippman [in collaboration with D. Zamir, Hebrew University, Israel]

Despite countless studies in multiple systems, the genetic and molecular underpinnings for how hybrid progeny outperform their parental inbreds (heterosis) have not been resolved. On the basis of classical studies in noncrop plants and recent findings in yeast, we have begun testing a new hypothesis that single-gene mutations can cause phenotypic overdominance in the heterozygous state through a reduction in gene dosage. By taking advantage of a large "mutant library" in an isogenic cultivated tomato background (*Solanum lycopersicum*, cv. M82), we have identified multiple genes that show heterosis through overdominance. However, the extent and manner by which single-gene mutations cause heterosis is unknown. We have used a robust phenomics platform to resolve the genetic and developmental basis of heterosis in two mutants for which we have

already identified the underlying genes. Surprisingly, we have discovered that *SINGLE FLOWER TRUSS* (*SFT*), which is the genetic originator of the flowering hormone florigen that drives the reproductive transition in all plants, increases yield up to 60% when loss-of-function alleles are heterozygous. Yield overdominance from *sft* heterozygosity is robust, occurring in distinct genetic backgrounds and environments, and we show that several traits integrate pleiotropically to drive heterosis in a multiplicative manner. Our results suggest that a simple reduction in the dosage of a single gene can cause dramatic transgressive variation. These findings provide the first example of a single overdominant gene for yield and suggest that screening for mutations with heterozygous effects on growth can improve productivity in other agricultural organisms.

On the basis of these findings, we are now extending our studies to screen for other mutants that show heterosis in the heterozygous state. Furthermore, because of the greatly reduced genomic complexity of single-gene heterosis, we are performing targeted and global quantitative gene expression analyses to link heterotic effects with transcriptional changes in developmental pathways and/or larger transcriptional networks.

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Soonju Park, Zach Lippman

EPIGENETIC INHERITANCE AND REPROGRAMMING IN PLANTS AND FISSION YEAST

R. Martienssen J. Calarco, A.-Y. Chang, K. Creasey, P. Finigan, J.-J. Han, K. Hansen, D. Irvine, A. Kloc, J. Le Pen, L. Lim, U. Ramu, M. Regulski, A. Schorn, R. Schwab, R. Shen, J. Simorowski, R.K. Slotkin, M. Tanurdžić, V. Thapar, F. van Ex, M.W. Vaughn, J. Xue, M. Zaratiegui

Plants and fission yeast exhibit a wealth of epigenetic phenomena, including transposon regulation, heterochromatic silencing, and gene imprinting, important both for plant breeding and for human health. They provide excellent model organisms to address the question of how epigenetic information is propagated to daughter cells. We are investigating the inheritance of heterochromatic silencing using the fission yeast *Schizosaccharomyces pombe* and the model plant *Arabidopsis thaliana* using a variety of genetic and genomic approaches. We discovered that fission yeast RNA interference (RNAi), which is required for transcriptional silencing of centromeric heterochromatin, occurs during S phase, allowing for copying of heterochromatic histone modifications following DNA replication and cell division. We also showed that in plants, cells in culture and male germline cells undergo massive epigenomic changes correlated with the appearance of a novel class of 21-nucleotide small interfering RNA (siRNA) from transcriptionally reactivated transposable elements (TEs). On the basis of our discovery of mobile endogenous small RNA signals in plants, we propose a model for the role of programmed TE reactivation in germline companion cells as part of a developmental mechanism for first revealing and then silencing TEs via small RNA. This year, we welcomed postdocs Kate Creasey, Andrea Schorn, Fred van Ex, Chunlao Tang, and URP Lisa Lim. We said farewell to Watson School students Eyal Gruntman and Sarahjane Locke, and postdoc Rebecca Schwab. Anna Kloc graduated from the genetics program at SUNY Stony Brook for a postdoc at Yale, and Keith Slotkin accepted a position at Ohio State University.

Epigenetic Reprogramming and Small RNA Silencing of Transposable Elements in Pollen

R.K. Slotkin, F. van Ex, M.W. Vaughn, M Tanurdžić [in collaboration with J.D. Becker and J.A. Feijó, Gulbenkian Institute, Lisbon]

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 pollen from the model plant *Arabidopsis*, we have found that TEs are unexpectedly reactivated and transpose, but only in the pollen 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 and accumulate in sperm, suggesting that siRNA from TEs activated in the vegetative nucleus can target silencing in gametes (Fig. 1). We propose a conserved role for reprogramming in germline 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.

Control of Female Gamete Formation by a Small RNA Pathway

R.K. Slotkin, A. Schorn, K. Creasey [in collaboration with J.P. Vielle-Calzada, Langebio-Cinvestav Mexico]

In the ovules of most sexual flowering plants, female gametogenesis is initiated from a single surviving gametic cell, the functional megaspore, formed after meiosis of the somatically derived megaspore mother cell (MMC). Because some mutants and certain sexual species exhibit more than one MMC, and many others are able to form gametes without meiosis (by apomixis), it has been suggested that somatic cells in the ovule are competent to respond to a local signal likely to have an important function in determination. We have found the *Arabidopsis* protein ARGONAUTE 9 (AGO9) controls female gamete formation by restricting the specification of gametophyte precursors in a dosage-dependent, non-cell-autonomous manner. Mutations in *AGO9* lead to the differentiation of multiple gametic cells that are able to initiate gametogenesis. The AGO9 protein is not expressed in the gamete lineage; instead, it is expressed in cytoplasmic foci of somatic companion cells. Mutations

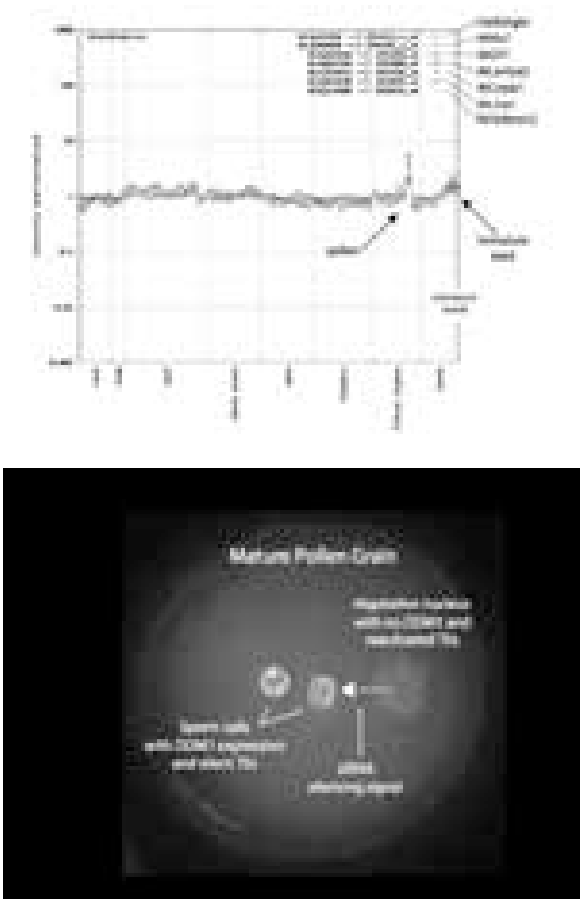

Figure 1. Epigenetic reprogramming and small RNA silencing of TEs in pollen. (*Top*) Expression of five different TEs, monitored with the Ath1 Affymetrix microarray, is restricted to pollen and immature seeds, as well as cultured cells (not shown). (*Bottom*) A model for small RNA signals in the pollen grain (stained with DAPI). TEs are expressed in the vegetative nucleus (*right*), which loses heterochromatin due to loss of the DECREASE IN DNA METHYLATION 1 (DDM1) protein. TE transcripts are rapidly converted into small RNA, which are translocated into sperm cells where they silence any TE active in the germline.

in *SUPPRESSOR OF GENE SILENCING 3* and *RNA-DEPENDENT RNA POLYMERASE 6* exhibit a defect identical to that of *ago9* mutants, indicating that the movement of small RNA silencing out of somatic companion cells is necessary for controlling the specification of gametic cells. AGO9 preferentially interacts with 24-nucleotide small RNAs derived from TEs, and its activity is necessary to silence TEs in female gametes and their accessory cells. Our results show that AGO9-dependent small RNA silencing is crucial to specify cell fate in the *Arabidopsis* ovule and that epigenetic reprogramming in companion cells is necessary for small RNA-dependent silencing in plant gametes.

Heterochromatin, Small RNA, and Postfertilization Dysgenesis in Allopolyploid and Interploid Hybrids of *Arabidopsis spp*.

M. Tanurdžić, P. Finigan, J. Le Pen, M. Vaugh [in collaboration with R.W. Doerge, Purdue University, and L. Comai, University of California, Davis]

In many plants, including *Arabidopsis*, hybrids between species and subspecies encounter postfertilization barriers in which hybrid seeds fail to develop or else give rise to infertile progeny. In *Arabidopsis*, some of these barriers are sensitive to ploidy and to the epigenetic status of donor and recipient genomes. Recently, a role has been proposed for heterochromatin in reprogramming events that occur in reproductive cells, as well as in the embryo and endosperm after fertilization. The 21-nucleotide siRNAs from activated transposable elements accumulate in pollen and are translocated from companion vegetative cells into the sperm (see Fig. 1); however, in the maturing seed 24-nucleotide siRNAs are primarily maternal in origin. Thus, maternal and paternal genomes likely contribute differing small RNAs to the zygote and to the endosperm. As heterochromatic sequences also differ radically between, and within, species, small RNA sequences will diverge in hybrids. If transposable elements in the seed are not targeted by small RNA from the pollen, or vice versa, this could lead to hybrid seed failure. Heterochromatin also has a role in apomixis and nucleolar dominance and may use a similar mechanism.

Endogenous *Trans*-acting siRNAs Mediate Non-cell-autonomous Effects on Gene Regulation in *Arabidopsis*

R. Schwab [in collaboration with O. Voinnet and M. Crespi, CNRS France]

Different classes of small RNAs refine the expression of numerous genes in higher eukaryotes by directing protein partners to complementary nucleic acids, where they mediate gene silencing. Plants encode a unique class of small RNAs, called *trans*-acting small interfering RNAs (tasiRNAs), which posttranscriptionally regulate protein-coding transcripts, as do microRNAs (miRNAs), and both small RNA classes control development through their targets. tasiRNA biogenesis requires multiple components of the siRNA pathway and also miRNAs. But although 21-mer siRNAs originating from transgenes can mediate silencing across several cell layers, miRNA

action seems to be spatially restricted to the producing or closely surrounding cells. We have previously described the isolation of a gene-trap reporter line for TAS3a, the major locus producing *AUXIN RESPONSE FACTOR (ARF)*-regulating tasiRNAs in the *Arabidopsis* shoot. Its activity is limited to the adaxial (upper) side of leaf primordia, thus spatially isolated from ARF activities, which are located in the abaxial (lower) side. We have shown by in situ hybridization and reporter fusions that the silencing activities of ARF-regulating tasiRNAs are indeed manifested non-cell-autonomously to spatially control ARF activities. Endogenous tasiRNAs are thus mediators of a mobile developmental signal and might provide effective gene silencing at a distance beyond the reach of most miRNAs.

The Maize Genome: Complexity, Diversity, and Dynamics

J.-J. Han, C. Tang, M. Regulski, M.W. Vaughn [in collaboration with D. Ware and W.R. McCombie, Cold Spring Harbor Laboratory, and the maize genome-sequencing consortium]

An improved draft nucleotide sequence of the 2.3-Gb genome of maize revealed more than 32,000 predicted genes. Nearly 85% of the genome is composed of hundreds of families of transposable elements, dispersed nonuniformly across the genome. By comparison with our previous genome sequencing by methylation filtration, we correlated methylation-poor regions with Mu transposon insertions, high gene density, and recombination. Careful annotation of a contiguous 22-Mb region of maize chromosome 4 revealed 84% of the sequence is composed of TEs nested within each other, of which most families are low-copy. 544 gene models were identified, as well as five miRNA genes. They include several candidates for the classical dominant *Tunicate1*, a pre-Columbian trait (podcorn) that maps to the interval and undergoes rearrangements and copy-number changes.

Mapping Epigenetic Mutations in Fission Yeast Using Whole-genome Next-generation Sequencing

D. Irvine, M. Zaratiegui, K. Hansen, A.-Y. Chang, M.W. Vaughn, L. Lim, A. Kloc [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory]

Fission yeast is an important model for epigenetic studies due to the ease with which genetic mutants can be isolated. However, it can be difficult to complement epigenetic phenotypes with genomic libraries in order to identify the genes responsible. This is because epigenetic phenotypes are typically unstable, and they can prohibit complementation if silencing cannot be reestablished. We have resequenced the fission yeast genome following mutagenesis to readily identify a novel mutant involved in heterochromatic silencing. Candidate genes were identified as functional single-base changes linked to the mutation, which were then reconstituted in a wild-type strain to recapitulate the mutant phenotype. By this procedure, we identified a weak allele of *ubc4*, which encodes an essential E2 ubiquitin ligase, as responsible for the *swi*603* mutant phenotype. In combination with a large collection of mutants and suppressor plasmids, next-generation genomic resequencing promises to dramatically enhance the power of yeast genetics, permitting the isolation of subtle alleles of essential genes, alleles with quantitative effects, and enhancers and suppressors of heterochromatic silencing. This provides a complementary strategy to analysis of the fission yeast deletion library, based on evolutionary prediction of genes involved in RNAi. We have also sequenced small RNA and other populations of small molecules from *S. pombe* and related species to investigate the evolution of small RNA silencing and heterochromatin in this model eukaryotic cell.

Enhancer-trap and Gene-trap Tagging of Transposons in *Arabidopsis*

J. Simorowski, U. Ramu, R. Shen

We have continued to grow our collection of gene-trap and enhancer-trap insertions in *Arabidopsis* and have now sequenced close to 40,000 insertion sites. Of recent interest, we have found insertions that monitor expression of TE in pollen and in RNAi-deficient ovules. We have also found enhancer-trap insertions that accurately report the expression of noncoding precursors of *trans*-acting siRNA, as well as genes required for cell–cell movement of RNA signals. These and many other individual lines have been distributed to other groups at CSHL, as well as to the community as a whole.

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Miguel Zaratiegui Biurrun

PLANT DEVELOPMENTAL GENETICS

M. Timmermans K. Bergami, D. Chitwood, C. Clendaniel, M. Dotto, C. Fernandez-Marco, C. Hu, A. Husbands, M. Lodha, K. Petsch, E. Plavskin, D. Skopelitis

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 Opposing Small RNAs

D. Chitwood [in collaboration with former postdoc F. Nogueira, University of Sao Paulo, Sao Paulo, Brazil]

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 21-nucleotide *TAS3*-derived *trans*-acting short interfering RNAs (tasiRNAs) (Fig. 1A). 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 establishment of organ polarity requires the precise spatiotemporal accumulation and relative efficacy of *TAS3*-derived tasiRNAs and miR166. To investigate the regulatory mechanisms that allow for the precise spatiotemporal accumulation of these polarizing small RNAs, we used laser-microdissection coupled to reverse transcriptase–polymerase chain reaction (RT-PCR) to determine the expression profiles of their precursor transcripts within the maize shoot apex.

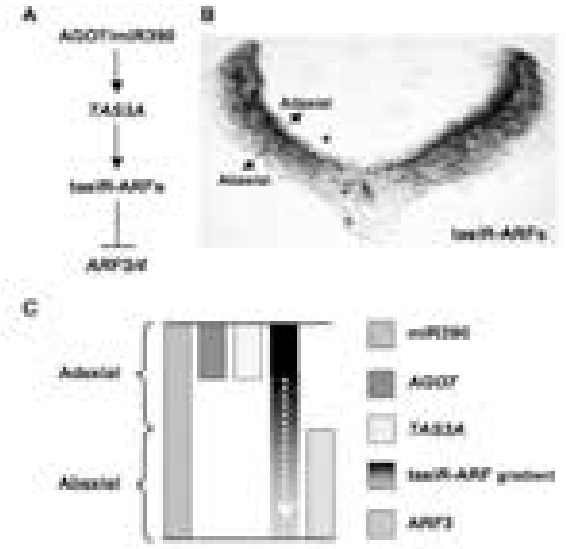

Figure 1. Pattern formation in leaves via small RNA mobility. (*A*) The tasiR-ARF pathway. (*B*) In situ hybridization using an LNA (locked nucleic acid) probe reveals a gradient of tasiR-ARF accumulation that is strongest on the adaxial (upper) side of the leaf and dissipates away toward the abaxial (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 most-adaxial 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 the mobility of this small RNA (*C*, dotted arrow). tasiR-ARFs act through a dose-dependent mechanism, such that this small RNA gradient creates a precisely defined domain of ARF3 expression and sharpens the adaxial–abaxial boundary.

Our data reveal that the pattern of mature miR166 accumulation results, in part, from intricate transcriptional regulation of its nine precursor loci and that only a subset of *mir166* family members contributes to the establishment of leaf polarity. We also showed that the polar expression of miR390 is established and maintained independent of the tasiRNA pathway. The comparison of small RNA localization data with the expression profiles of precursor transcripts suggests that miR166 and

miR390 accumulation is also regulated at the level of biogenesis and/or stability. Furthermore, *mir390* precursors accumulate exclusively within the epidermal layer of the incipient leaf, whereas mature miR390 accumulates in the subepidermal layers as well. Regulation of miR390 biogenesis, stability, or even discrete trafficking of miR390 from the epidermis to underlying cell layers provides possible mechanisms that define the extent of miR390 accumulation within the incipient leaf, which patterns this small field of cells into adaxial and abaxial domains via the production of *tas3*-derived tasiRNAs.

Pattern Formation via Small RNA Mobility

D. Chitwood [in collaboration with former postdoc F. Nogueira, and T. Montgomery, M. Howell, and 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 had 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 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.

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. Skopelitis, C. Hu

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 shoot apical meristem, whereas mature miR390 accumulation extends into the meristem proper. 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 it may be a general property of miRNAs expressed beneath the SAM.

To further investigate the possible movement of miRNAs in a variety of developmental contexts, we have generated artificial miRNAs against *PHYTOENE DESATURASE* (miR-PDS), whose silencing activity results in an easy observable photobleaching phenotype. When expressed from the epidermis, in a mutant background defective in production of secondary siRNAs, miR-PDS results in the silencing of *PDS* in subepidermal cell layers, consistent with small RNA mobility. Similarly, expression of a modified *TAS3* precursor in which the tasiR-ARFs are replaced by small RNA sequences targeting *PDS* (*TAS3-PDS*) from the epidermis results in a photobleaching phenotype. We are taking advantage of these genetic resources to analyze parameters of small RNA mobility, such as tissue specificity and direction

and range of movement, and whether this is dose dependent. We are also using stable uniform bleaching lines in forward genetic screens to identify factors involved in the cell-to-cell trafficking of miRNAs and tasiRNAs.

The Ancestral Role of the tasiRNA Pathway

E. Plavskin [in collaboration with R. Quatrano, Washington University, St. Louis, Missouri]

Genes involved in tasiRNA biogenesis, as well as miR390 and the *TAS3* loci themselves, are conserved in the moss *Physcomitrella patens*. Even more interesting, tasiRNAs produced from *Physcomitrella TAS3* loci target transcripts of *AUXIN RESPONSE FACTOR* genes. Early divergent 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* tasiRNA pathway will thus be distinct from its known role in adaxial–abaxial patterning of leaves in flowering plants. To dissect the developmental processes in moss regulated by tasiR-ARFs, we are characterizing knockouts of genes involved in tasiRNA biogenesis in *Physcomitrella*, as well as the targets of the moss tasiR-ARFs. We have shown that *sgs3* loss-of-function mutants display a branching defect in the filamentous stage of growth. These mutants also appear to undergo a precocious transition from filamentous growth to budding—reminiscent of the early transition from juvenile to adult growth seen in *Arabidopsis* tasiRNA biogenesis mutants. A knockout of *PpARF2*, one of the targets of the miR390-dependent tasiRNAs, was found to be lethal, which might reflect a defect in branch formation. Using a transcriptional reporter, we have found that the *MIR390b* precursor is expressed strongly in the protonema. Reporter constructs to monitor expression of the other two *MIR390* precursors, additional tasiRNA biogenesis components, and the *ARF* targets are being generated, as well as mutants that express tasiRNA-insensitive versions of the *ARF* targets. Analysis of these *Physcomitrella* mutants and reporter lines will shed light on the ancestral role of this highly conserved small RNA pathway in plant development.

The START Domain Regulates HD-ZIPIII 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. Such mutant transcripts accumulate on both sides of developing leaf primordia resulting in their adaxialization. HD-ZIPIII proteins contain a predicted START lipid/ sterol-binding domain, suggesting that HD-ZIPIII activity may further be regulated by an unknown ligand. To assess whether the START domain is important for PHB function, we generated transgenic *Arabidopsis* lines that express START domain variants of a PHB-YFP fusion protein. As recessive loss-of-function *phb* mutants display no distinguishing phenotypes, these PHB-YFP reporters also carry silent mutations in the miR166 target site. As expected, nearly all T1 transformants expressing the miR166-insensitive PHB-YFP fusion develop severely adaxialized leaves. Expression of a PHB-YFP variant, in which two highly conserved arginine residues in the START domain are mutated to leucine, yielded plants with less severe leaf phenotypes. Transgenic lines expressing a PHB-YFP variant with a deletion of the START domain resemble wild-type plants.

Confocal imaging revealed that the START-deleted PHB-YFP fusion protein, like normal PHB, localizes to the nucleus. This indicates that the START-deleted PHB-YFP protein is not inherently unstable and that the START domain is critical for PHB function. Moreover, these data argue against a role for the START domain in regulating the subcellular localization of PHB. We are currently investigating other potential mechanisms through which the START domain may regulate PHB activity, such as DNA-binding affinity, activation of transcription, or interaction with required protein partners. Preliminary data using a modified yeast–one-hybrid approach suggest that the START domain may be dispensable for transcription activation in yeast. These yeast strains will also be used to screen a 380,000 small-molecule library for putative agonists/antagonists of PHB and other HD-ZIPIII proteins in collaboration with partners at the Memorial Sloan-Kettering Cancer Center.

Characterization of Novel tasiRNA Loci in Maize

M. Dotto [in collaboration with M. Aukerman, M. Beatty, and R. Williams, DuPont-Pioneer, Wilmington, Delaware]

tasiRNAs are processed from noncoding *TAS* precursor transcripts. Following cleavage by a miRNA-loaded ARGONAUTE complex, one of the cleavage products is converted into double-stranded RNA (dsRNA) through the activities of RDR6 and LBL1/SGS3 and

subsequently processed by DCL4 into phased 21-nucleotide siRNAs. *Arabidopsis* contains four characterized *TAS* gene families. AGO1 is required for the biogenesis of tasiRNAs from the *TAS1*, *TAS2*, and *TAS4* loci, whereas miR390-loaded AGO7 triggers the biogenesis of *TAS3* tasiRNAs. We previously identified four *TAS3* loci in the maize genome; however, the *TAS1*, *TAS2*, and *TAS4* loci are not conserved between *Arabidopsis* and maize. To identify potential novel *TAS* loci in maize, we are using a deep-sequencing approach to compare the small RNA content between wild-type and *lbl1* mutant apices. In addition, we are characterizing small RNAs that associate with specific AGO proteins. The maize genome encodes 20 AGO proteins, including one AGO7 and four AGO1 homologs. We are generating transgenic maize plants expressing FLAG-tagged versions of ZmAGO7 and ZmAGO1b to isolate associated small RNAs via immunoprecipitation. We expect to identify novel tasiRNAs, miRNAs, and possibly the transcripts that are the targets of these small RNAs. For additional information regarding the small RNA pathways in maize, we are also characterizing small RNAs that associate with ZmAGO10. This AGO protein has been implicated in the miRNA and tasiRNA-mediated regulation of meristem maintenance and leaf development in *Arabidopsis.*

Regulation of Maize Leaf Determination and Patterning by *Laxmidrib1*

M. Dotto [in collaboration with B. Lin, J. Jaqueth, and K. Fengler, DuPont-Pioneer, Wilmington, Delaware]

Laxmidrib1-O (*Lxm1-O*) is a dominant ethylmethanesulfonate (EMS)-induced mutation in maize that causes pleiotropic developmental phenotypes that vary depending on inbred background. The most-penetrant defect is the presence of a flexible midrib. In addition, in *Lxm1-O* mutants, the blade/sheath boundary is displaced distally into the leaf blade, a phenotype reminiscent of that caused by ectopic *KNOX* expression. In the W22 inbred, *Lxm1-O* also causes the partial adaxialization of leaves. These observations indicate a role for *Lxm1* in leaf determination and patterning. To further characterize the role of *Lxm* in these processes, we analyzed its interaction with *rs2*, *Rld1-O,* and *lbl1*. Like *Lxm1-O*, mutation of the *myb* domain protein RS2 leads to ectopic *KNOX* expression in developing primordia, resulting in the formation of leaves with a characteristic rough sheath phenotype. Surprisingly, in *Lxm1-O rs2* double mutants, this sheath phenotype is nearly completely suppressed, whereas the *Lxm1* blade phenotype is slightly enhanced. In maize, abaxial–adaxial patterning requires the activity of *Rld1* and *lbl1*, which encode an HD-ZIPIII transcription factor and a homolog of the tasiRNA biogenesis component SGS3, respectively. *Rld1-O* single mutants develop adaxialized leaves due to ectopic expression of *Rld1* caused by insensitivity to miR166 regulation. *Lxm1-O* and *Rld1-O* enhance each other, resulting in the formation of severely adaxialized leaves. In contrast, *Lxm1-O* suppresses the partial abaxialized leaf phenotypes of weak *lbl1* mutants. Efforts to clone *Lxm1* by a map-based approach are ongoing. *Lxm1* maps to a region spanning 200 kb on chromosome 3.

Small RNA-regulated Gene Networks in SAM Function and Adaxial–Abaxial Patterning

K. Petsch [in collaboration with M. Scanlon, Cornell University, Ithaca, New York; D. Janick-Buckner, Truman State University, Kirksville, Missouri; G. Muehlbauer, University of Minnesota, Minneapolis; J. Yu, Kansas State University, Manhattan; P. Schnable, Iowa State University, Ames]

Mutations that block tasiRNA biogenesis in maize lead to the formation of abaxialized leaves. To gain insight into gene networks regulated by tasiRNAs and/or involved in adaxial–abaxial patterning, we used laser capture microdissection (LCM) coupled with microarray analyses to compare the global gene expression profiles in the SAM and the first leaf of wild-type and *lbl1* mutants. We identified 352 genes whose expression is significantly altered in *lbl1*. Interestingly, 47 of these genes also show differential expression between the incipient leaf and the SAM proper. In situ hybridization analysis identified two potential new polarity factors among the subset of genes enriched in the incipient leaf. *seven-absentia-like* is expressed adaxially and shows reduced expression in *lbl1* mutants, whereas expression of a *squamosa promoter binding protein-like* is enriched in the abaxial domain of normal leaf primordia and accumulates ectopically in *lbl1* mutants.

We are currently screening various reverse genetic resources available in maize for mutations in selected genes differentially expressed in *lbl1*. Mutant alleles have been identified for a SCARECROW-like protein related to *AtSCL9/14*, *rdr6*, a putative B3 DNA-binding domain containing protein and *arf3a*. These mutants are currently under analysis for defects in adaxial–abaxial patterning and SAM function. To further supplement our analysis of genes involved in the

regulation of adaxial–abaxial patterning, we are also analyzing several *dicer-like4* (*dcl4*) mutants. Both *lbl1* and *dcl4* function in the same tasiRNA pathway and are single-copy genes. However, severe alleles of *lbl1* result in embryo lethality, whereas null mutations in *dcl4* show phenotypic resemblance to the weak *lbl1-ref* allele. The possibility that another DICER family member can partially compensate for the loss of *dcl4* activity is currently being tested. This project will provide novel insights into the gene networks controlled by tasiRNAs and their contribution to organ polarity.

Establishment of Determinacy during Organ Development

M. Lohda, C. Clendaniel

Stem cell activity in the SAM is maintained in part by the class I *KNOX* homeobox genes. To give rise to differentiating structures, such as leaves, *KNOX* gene expression needs to be maintained in a stable "off" state throughout lateral organ development. We have previously shown that this process is mediated by the transcription factors AS1 and AS2, which recruit the chromatin-remodeling factor HIRA to the *KNOX* loci. We have now identified a role for a polycomb repressive complex 2 (PRC2) in the stable silencing of *KNOX* genes during leaf development. PRC2 complexes have histone H3K27 trimethylation (H3K27me3) activity, and we have identified this repressive mark on nucleosomes at the *BP* and *KNAT2* loci in leaves of *Arabidopsis*. Besides, mutations in the PRC2 component CLF lead to a reduction in the levels of this H3K27me3 mark at the *KNOX* loci and the ectopic expression of *KNOX* genes in developing leaves. Moreover, we have shown that in *as1 hira* mutants, the levels of H3K27me3 are significantly reduced, consistent with a role for the AS1-AS2-HIRA complex in the recruitment of PRC2. To assess this possibility, we have developed tagged lines for the PRC2 components MSI1 and CLF, and raised monoclonal and polyclonal antibodies against HIRA. These resources are being used to monitor recruitment of PRC2 and HIRA to the *KNOX* loci in wild-type and *as1*, *as1 hira*, and *clf* mutant seedlings. To determine whether the AS1-AS2-HIRA complex can interact directly with PRC2 components, we have used biomolecular fluorescence complementation (BiFC). This revealed a physical interaction between HIRA and MSI1. These data suggest that cellular differentiation is achieved via an epigenetic mechanism in which HIRA serves as an intermediary factor that recruits PRC2 to pluripotency determinants. HIRA and PRC2 have also been implicated into the regulation of stem cell homeostasis in mammals, such that our findings in plants may provide an excellent framework for future studies on HIRA- and PcG-mediated gene silencing in mammalian embryonic stem cells and induced pluripotent stem cells.

Characterization of *chups*, an Embryonic Patterning Mutant in *Arabidopsis*

C. Fernandez-Marco, K. Bergami

Embryogenesis in *Arabidopsis* proceeds through a series of stereotypic divisions. An asymmetric division of the zygote gives rise to the apical embryonic lineage and the basal extra-embryonic suspensor. After several more rounds of cell division, the upper tier of the embryo proper generates the shoot apical meristem, whereas the upper cell of the suspensor undergoes two successive asymmetric divisions to give rise to the root apical meristem. We have identified a mutant that delays embryonic development. At the time of fruit dehiscence, mutant embryogenesis has progressed to just the late globular or heart stage. We have called this mutant *chupa chups* (*chups*), which means lollipop in Spanish. Despite the delay in embryogenesis, patterning of the shoot apical meristem is unaffected in *chups* mutants. In contrast, cell division of the hypophysis, the specialized cell of the suspensor that ultimately gives rise to the quiescent center of the root stem cell niche, is perturbed in *chups*. Characterization of molecular markers suggests that auxin transport is altered in *chups*. As a result, an auxin maxima is no longer established at the boundary between the proembryo and suspensor, leading to a failure to specify the hypophysal cell. *chups* mutant embryos can be rescued by transferring fertilized ovules onto culture medium. Consistent with the above-mentioned patterning defects, rescued mutant seedlings develop a normal shoot but lack a root. We are currently using map-based and next-generation sequencing approaches to clone the *chups* mutation. The outcome of these experiments will allow us to position CHUPA CHUPS in the genetic network that patterns the root stem cell niche during embryogenesis.

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Christina Fernandez Marco

BIOINFORMATICS AND GENOMICS

CSHL is at the leading edge of efforts to manage and interpret the massive amounts of information generated by biological experiments, across fields. Recently, the Laboratory took a further step into this realm by creating a Center for Quantitative Biology. The new center reflects the importance of math and statistics in the forging of new approaches to problems and new ways of understanding the results of contemporary experimentation.

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 a decade ago. They have brought online a new generation of Solexa sequencers and optimized their function to a level at which 10 billion DNA bases can be sequenced in a typical day, and on some days, as many as 20 billion. McCombie's team took part in the effort, completed this year, to sequence the genome of maize, one of the world's most important crops. They have also had an important role in projects to sequence the flowering plant *Arabidopsis thaliana*, the fission yeast *Schizosaccharomyces pombe,* and *Homo sapiens*. New work is 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 Sloan-Kettering Cancer Center, they are using a method called hybrid resequencing, developed with Greg Hannon, to look at mutations in samples collected from patients with prostate cancer.

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. They have recently established the modENCODE Data Coordination Center, which is responsible for collecting, integrating, and publishing the information collected by the ENCODE (ENCyclopedia of DNA Elements) consortium in a form that can be extended and combined with information from other human and model organism genome databases. The lab has also had an important role in the HapMap project, a database of human single-base-pair variations that provides information on the genetic variability of 11 human populations. Stein's group also manages and curates the WormBase and Reactome databases and is involved in 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 the evolution of genome sequences in plants. By looking comparatively across the genomes of plants in the same lineage, they seek answers to such questions as: how are genes conserved and lost over time and what are the fates of duplicated genes? Ware's team also studies gene regulation in plants, looking at *cis*-regulatory elements and characterizing microRNA genes and their targets, with the objective of understanding how these parts of the plant genome work together to determine spatial and temporal expression of genes. The lab had an important role in the 4-year project to produce a reference genome of maize, providing annotations for the genome and helping to generate a draft haplotype map, a gauge of maize's genetic diversity. They have devoted special attention to examining diversity in maize and grape, with the ultimate aim of accelerating development of strategies to introduce new germplasm, needed to meet the demands of an increasing population and a changing environment. They have also brought fully sequenced genomes into an integrated data framework, to enhance the power of their comparative studies; it now includes the genomes of *Arabidopsis*, maize, rice, sorghum, grape, and poplar.

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.

DEVELOPMENT OF NEXT-GENERATION SEQUENCING TECHNOLOGY

W.R. McCombie J. Busuttil, L. Cardone, T. Caulfield, M. Chen, E. Ghiban, M. Gierszewska, I. Grabill, M. Kramer, S. Lata, D. Lewis, S. Mavruk, P. Mocombe, S. Muller, M. Ossipov, J. Parla, D. Rebolini, L. Spiegel, J.C. Yao

Expanding Our Ability to Examine Genome Variation

Much of our activities in 2009 were focused on building an infrastructure that would allow large-scale studies with next-generation sequencing. To do this, we made improvements in both the wet lab and computational segments of the process. For the wet lab processes, we expanded the number of Illumina sequencers and staff. This required an extensive effort in training and focus on quality control. Ms. Laura Cardone was made manager of the sequencing group and led the training efforts. For the informatics arena, we streamlined the analysis pipeline and trained additional staff in its operation. The software provided by Illumina for analysis of data from the instruments underwent several revisions during 2009. These improvements, coupled with better chemistry, gave a dramatic increase in instrument throughput and quality. Figure 1 shows the monthly output in sequences for all projects at CSHL.

In addition, in late 2009 we began exploring ways to make these capabilities further available to the CSHL community. The main roadblock to this is the amount of computational analyses required. We are testing several options and have as a goal for 2010 the implementation of a Lab-wide infrastructure to support the bioinformatic analysis of next-generation sequence data.

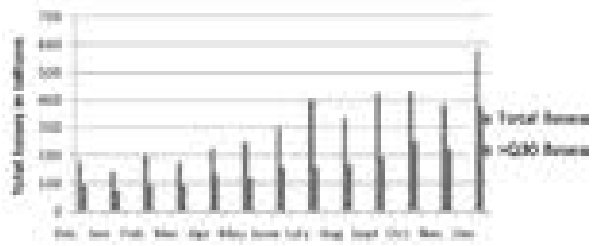

Figure 1. Comparison of total bases generated versus total high-quality bases for monthly Illumina runs from December 2008 through December 2009. We use the quality measure of Q30 to indicate a very-high-quality sequence for the purpose of pushing the limits on quality. Much-lower-quality bases are still quite useful, but focusing on the more stringent Q30 metric provides constant pressure to improve production quality.

Family-based Analysis of Bipolar Disorder by Whole-genome Resequencing

This work was done in collaboration with D. Blackwood and D. Porteous (University of Edinburgh).

We are studying a large Scottish pedigree where many family members have bipolar disorder or recurrent major depressive disorder. A genetic study of this family by our collaborators at the University of Edinburgh suggested that a 20-Mb critical region on chromosome 4 has significant linkage to bipolar disorder (Le Hellard et al., *Biol Psychiatry 61:* 797–805 [2007]).

We decided to use next-generation sequencing (Illumina Genome Analyzer II [GAII]) to resequence the entire genome of individuals from this family, focusing analysis on the 20-Mb critical region on chromosome 4. We hope to discover rare variants associated with bipolar disorder susceptibility. We completed the sequencing of two individual samples from this family using paired-end 101-cycle and 125-cycle runs and achieved ~21x–25x coverage on each individual. Reads from all flow cells for each individual sample were then aligned to UCSC hg18. We processed the mapping and single-nucleotide polymorphism (SNP) calling by using the Burrows–Wheeler alignment (BWA) and sequence alignment map (SAM) tools (Li et al., *Bioinformatics 25:* 2078–2079 [2009]) pipeline. Potential polymerase chain reaction (PCR) duplicates were removed, and error-prone variant calls were then filtered out by using a minimum consensus quality score of 20 and a minimum read depth of 20x. As expected, ~3–4 million SNPs were found in each individual. However, there were fewer SNPs in common between these two individuals and also not present in the public SNP database. In fact, a relatively small number of variants were common in both affected individuals, not present in the SNP database and in coding exons of the critical regions.

We are currently extending this study to another affected individual and two unaffected family members to further genetically refine possible bipolar-associated vari-

ants. The sequence data will be validated by a variety of techniques, including comparison of whole-genome sequencing by high-density genotyping (Illumina-IVb), and selected variants will be validated using Sanger sequencing. The resulting studies will enhance our understanding of molecular mechanisms of the cause and development of bipolar disorder and further lead to novel diagnosis and treatment of this psychiatric disorder.

Targeted Resequencing of the Human *DISC1* Gene Using Long-range PCR and Illumina Sequencing

This work was done in collaboration with D. Blackwood, D. Porteous, and I. Deary (University of Edinburgh) and P. Visscher and A. McRae (University of Queensland).

Disrupted in schizophrenia-1 (*DISC1*) is a strong candidate gene for association with schizophrenia and other major psychiatric disorders. With our collaborators in Edinburgh and Queensland, we are determining variation in the *DISCI* region in ~1000 affected patients and 1000 controls. To resequence the *DISC1* gene, we designed primers to generate 4–11-kb amplicons to tile across an ~523-kb region of chromosome 1 containing *DISC1*. The scope of this project encompasses 2210 samples, which can be divided into groups of individuals diagnosed with schizophrenia, bipolar disorder, unipolar disorder, or control individuals not diagnosed with psychiatric illness. We were able to ultimately scale our throughput up to 100 samples amplified per week in order to complete our *DISC1* amplifications in about 1 year. During 2009, we successfully amplified 1508 of the remaining 1533 samples to be amplified from our 2210 sample set (Table 1). This was accompanied by the sequencing of the newly amplified samples using paired-end 36-cycle Illumina sequencing runs. To enable higher throughput during sample sequencing, we developed custom sequencing adaptors to append molecular bar codes onto each sample library prior to sequencing. Our custom bar codes consist of a unique string of five bases that are present at the beginning of each sequence read. The use of our bar codes for this project allowed us to multiplex up to six individuals per lane of Illumina sequencing, thus greatly reducing cost and increasing the speed of the project.

To analyze the *DISC1* data generated by our Illumina sequencers, we used a Perl script to split the sequence reads according to bar-code sequence and used ELAND software (included in the Illumina analysis pipeline) to align the reads from each sample to our *DISC1* target region. The coverage of this region at 30X or greater sequencing depth was used to qualify our data for subsequent SNP analysis, for which we required an 80% cutoff per sample (Table 1). For SNP identification, we used the Maq software written by Heng Li (*Genome Res 18:* 1851–1858 [2008]).

Table 1. Overview of Samples Resequenced in our DISC1 Study during 2009

Total samples in study	2210
Samples amplified and sequenced	1508
Range of coverage at ≥30x	0–99%
Average coverage at ≥30x	79%

On the basis of the results of our first pass attempt to PCR-amplify and resequence *DISC1*, we identified 463 samples that did not meet our 80% coverage at ≥30x cutoff for SNP analysis. Subsequently, we initiated a second round of resequencing for these samples, which involved a few instances of repeated PCR amplification of *DISC1*, but mostly just resequencing of the original *DISC1* amplicons generated from these samples.

For samples that did obtain adequate coverage during our first round of *DISC1* resequencing, we proceeded to validate identified SNPs using capillary sequencing (ABI 3730), starting in December of 2009. Our initial validations revealed a high rate of false positives that were likely introduced during sample preparation. Interestingly, virtually all of these patients have been genotyped, so we have many thousands of SNPs to examine for false negatives, and this rate is quite low. So it appears that we are introducing a small number of variants into the process and then effectively detecting them. We are continuing with various validation studies to identify the major source of false positives and are working on revising our validation plan based on our ongoing results.

Targeted Resequencing of *DTNBP1* Using Long-range PCR and Illumina Sequencing

This work was done in collaboration with A. Malhotra and T. Lencz (Zucker Hillside Hospital).

Variants in the *DTNBP1* (dystrobrevin-binding protein-1) gene are associated with bipolar disorder and may also be associated with modulating effects in schizophrenia. Our collaborators at the Zucker Hillside Hospital provided 70 schizophrenia patient samples and controls to be sequenced. PCR primers were designed to tile overlapping amplicons ranging from ~2 to 10 kb across the ~140-kb *DTNBP1* gene region

with 1–2 kb flanking upstream and downstream. The samples were whole-genome-amplified and then PCR-amplified. Of the 70 samples, 68 were successfully PCR-amplified and sequenced on the Illumina instruments. The samples were multiplexed with our custom bar-code adaptors so that three to six samples could be sequenced per lane. The samples were sequenced with paired-end 36-bp runs, and the bar codes in each lane were deconvoluted with a Perl script. Individual samples were then aligned to the *DTNBP1* reference sequence (downloaded from UCSC hg18) and coverage was assessed. Samples with <90% of the region covered at 30-fold depth or better were sequenced again to improve coverage. We obtained >90% coverage at the required depth on 59 of the 68 samples. The samples were then analyzed for variants. Alignment and SNP calling was performed with Maq (Li, *Genome Res 18:* 1851–1858 [2008]). Duplicate read pairs (which are likely PCR artifacts) were removed, and the SNP calls were then filtered for a minimum consensus quality score of 20 and minimum coverage depth of 20-fold. The SNP call data for all patients were then sent to our collaborators at Hillside, who chose 773 variants for further validation. Variants were chosen for validation based on the following criteria:

1. Annotated and unannotated variants that differ significantly between risk haplotype carriers and noncarriers.
2. Exonic variants and variants within 100 bp upstream and downstream from the exons.
3. Variants in the 3′ and 5′ untranslated regions.
4. Variants that are potentially triallelic.
5. Nonannotated variants with an MAF >0.05.

Validation efforts for the selected variants are now in progress using Sanger sequencing on ABI 3730 capillary sequencing instruments.

Sequencing from Mixed Prostate Cancer Cell Populations with the Illumina Platform

This work was done in collaboration with C. Sawyer, H. Scher, and D. Danilla (Memorial Sloan-Kettering Cancer Center).

Next-generation sequencing has opened new opportunities in cancer genomics. One important consideration in the study of cancer is the fact that many tumors are very heterogeneous and contain multiple cell types. We are interested in evaluating our ability to determine the minimum percentage of neoplastic cancer cell types that must be present in order to be detected in a background of normal DNA and sequencing errors.

To test the limits of detection on the Illumina GAII sequencing platform, our collaborators from Memorial Sloan-Kettering Cancer Center provided sorted cell mixtures of LnCap and VCap human prostate cancer cell lines. The samples ranged from pure LnCap and pure VCap samples (0, 5, 10, 20, 50, and 100 cells each) to mixtures of the two cell types (cell ratios of 0/50, 10/40, 25/25, and 50/50). PCR primers were chosen to tile across the *p53* gene, and each of the cell populations was PCR-amplified and then sequenced on the Illumina GAII. The samples were bar coded and pooled so that two samples were sequenced per lane. The bar codes were then sorted with a Perl script, and the sequence reads for

Table 2. Examples of Allele Frequency Detected in the Mixed Cell Populations

p53 position	Sample	Ref Base	Genotype	A	C	G	T	NRAF
16917	LNCaP 50	C	C/T	3	1368	3	1863	
	VCaP 50	C	C	3	3899	3	3	
	LNCaP/VCaP 0/50 C5	C	C	6	2329	1	1	
	LNCaP/VCaP 10/40 E4	C	C/T	1	1784	2	505	
	LNCaP/VCaP 25/25 D3	C	C/T	1	2733	1	1221	
	LNCaP/VCaP 50/50 C6	C	C/T	1	1413	2	1710	
	LNCaP/VCaP 40/10 E2	C	C/T	1	1918	0	1131	
	LNCaP/VCaP 50/0 D1	C	C/T	0	1480	4	1569	
	LNCaP/VCaP 50/0 E1	**C**	**C/T**	**4**	**12898**	**9**	**386**	**NRAF only 3%**
25889	LNCaP 50	G	A/G	483	0	320	0	
	VCaP 50	G	G	2	0	479	0	
	LNCaP/VCaP 0/50 D5	G	G	2	0	593	1	
	LNCaP/VCaP 10/40 D4	G	A/G	66	0	226	1	
	LNCaP/VCaP 25/25 D3	G	A/G	333	2	316	1	
	LNCaP/VCaP 40/10 C2	**G**	**A/G**	**72**	**0**	**641**	**0**	**NRAF only 10%**
	LNCaP/VCaP 50/0 D1	G	A/G	537	1	273	0	
	LNCaP/VCaP 50/0 E1	**G**	**A/G**	**95**	**0**	**1091**	**0**	**NRAF only 8%**
	LNCaP/VCaP 50/50 C6	G	A/G	335	0	350	0	

each sample were aligned to the *p53* reference (UCSC hg18) using the Illumina ELAND alignment software. SNPs were then called using the SnpFinder program (Zhenyu Xuan, CSHL). The SnpFinder program uses a heuristic method that counts alleles present in the underlying reads mapped to each position to determine regions where the alleles differ from the reference.

Three SNP positions where a variant was clearly present in the pure LnCap but not the pure VCap cells were analyzed to determine the allele frequency of the nonreference allele in the mixed cell populations (see Table 2, on previous page).

Although the nonreference allele was detected in the mixed cell populations, it was not always present at what we would typically consider significant levels (minimum 20% minor allele frequency for a heterozygote). We will use this information and expand this study to develop models for SNP detection in heterogeneous samples.

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E. Ghiban

GENOME-SCALE DATABASES OF PATHWAYS, GENETIC VARIATIONS, AND EVOLUTION

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

I have moved the bulk of my laboratory to the Ontario Institute for Cancer Research in Toronto, Ontario, but I still maintain a small group at CSHL working on the iPlant Collaborative project.

The iPlant Collaborative is a virtual organization of faculty, students, postdoctoral fellows, staff, high-performance computing experts, and professional software developers drawn from CSHL, the University of Arizona, and the Texas Advanced Computer Center. iPLant is designed to solicit and meet biological grand challenge questions from the plant science community and, in close consultation with this community, create a cyberinfrastructure that makes it possible to meet these challenges. In 2009, two self-forming grand challenge teams were chosen by a community representative Board of Directors to work with iPlant personnel to develop "Discovery Environment (DE)," a cyberinfrastructure and user interface to help with the biological grand challenges.

In 2009, our group took on leadership of the engagement team or the iPlant Tree of Life (iPToL) Grand Challenge Project. The fundamental aim of this project is to assemble a comprehensive phylogenetic tree of half a million known species of green plants for better knowledge of evolutionary relationships, which will yield new insights across the plant sciences. The engagement team is composed of team leader plus 10 analysts and developers and interfaces directly with the working group leaders and plant science community members to develop prototype software and analyze design requirements for the iPlant core software development team. The scientific leader of this team and three of the analysts are based at CSHL.

Collaborative implementation of the grand challenge is organized into working groups with focused development goals. The four main working groups are Big Trees, Data Assembly, Tree Reconciliation, and Trait Evolution. Two cross-cutting working groups to develop shared data and compute infrastructure are Data Integration and Tree Visualization.

Development efforts made in collaboration with these working groups in 2009 will combine to result in an early 2010 release of the iPToL discovery environment, which is a web-based application that will be a portal for the green plant tree of life, for visualization, browsing, and analysis.

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PLANT GENOME ORGANIZATION, EVOLUTION, AND FUNCTION

D. Ware S. Avraham, J. Chia, A. Chuah, A. Eveland, M. Levy, Z. Liu, J. Lu, S. Kumari, A. Olsen, S. Pasternak, L. Ren, W. Spooner, J. Stein, J. Thomason, P. Van Buren, S. Wei, K. Youens-Clark, L. Zhang

Our lab conducts collaborative research in the field of plant genomics, with both basic and applied objectives. In the next 50 years, grain yields will need to double to match population growth—in a changing climate, with fewer inputs of water and fertilizer, and on increasingly marginal lands. Plant biology is poised to make significant contributions to meet these needs, as well as the growing demand for renewable energy resources. Application of multidisciplinary research with a basis in genomics will contribute to our understanding of complex traits and help drive a second "green revolution." Ongoing activities in the last year supported bioinformatics approaches to manage, analyze, and visualize relationships between sequence and phenotype data. The resulting resources of sequences, gene annotations, variation data, expression profiles, standards, and software will support future experiments in the laboratory as well as the field.

Maize Genome Sequencing Project

Our group has led data coordination, analysis, and visualization efforts as part of the Maize Genome Sequencing Consortium, a 4-year, multi-institutional project funded by the National Science Foundation (NSF). The project seeks to sequence the *Zea mays* B73 genome. Over the course of the project, our group has developed and refined a comprehensive annotation pipeline, based on standard bioinformatics tools provided by the Ensembl project. The pipeline is now a central resource for all plant genome assemblies important for various distinct projects in our lab, such as Gramene and OMAP (the Oryza Map Alignment Project). We also develop, run, and support MaizeSequence.org, the official public portal of the maize project. The site provides a maize genome browser, research tools, and full access to the maize data and tightly integrates with other online resources, such as Gramene. 2009 marks an exciting fourth and final year of the project. On the basis of the complete sequencing effort of the maize genome, an initial attempt at a genome assembly, to which our group has contributed significant effort, has been released. We have collaborated with various researchers to analyze and annotate the maize genome (Fig. 1) and provide, among others, a reliable set of 32,540 ordered genes. All of the data that we collected from our collaborators are now accessible through our web portal www.maizesequence.org and as part of a *Science* publication in November.

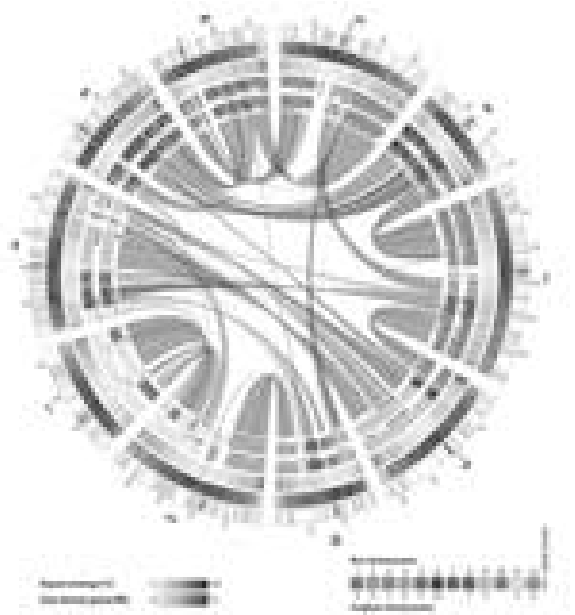

Figure 1. Overview of the maize B73 genome, showing 10 chromosomes. Concentric plots, from outer to inner rings: physical map, repeat density, gene density, synteny with sorghum, and synteny with rice. Genes and repeats form density gradients that are negatively correlated along centromere-telomere axes. Duplicate regions formed from polyploidy are indicated with ribbons.

Consequences of Ancient Tetraploidy in Maize

Whole-genome doubling (polyploidization) is a predominant mode of speciation in flowering plants. In both monocots and eudicots, recurrent rounds of polyploidy are thought to have provided raw material for the evolution of new traits and possibly contributed to the extraordinary adaptive radiation of these groups. We are studying how ancient tetraploidy in the maize lineage shaped the structure and function of its genome. Although highly rearranged, orthologous regions covering ~90% of the B73 assembly were mapped to rice and sorghum, grasses that diverged from maize prior to duplication. Using this information, we reconstructed remnants of maize's two ancestral subgenomes (designated A and B), which were likely brought together by interspecies hybridization some 5–12 million years ago. Although loss of redundant duplicate genes is a common outcome of whole-genome duplication, an unanticipated finding was a strong imbalance of gene loss between subgenomes. We estimate that the A parent lost ~23% of its genes, compared to ~56% of genes lost in the B parent. Thus, the current gene complement of maize is dominated by the contributions of one parent over the other. Greater than 8000 genes were retained as duplicates in both subgenomes, suggesting that they have essential, rather than redundant, function. Analysis of Gene Ontology terms showed a statistically significant enrichment for genes with regulatory function, in particular transcription factors. In a separate study, we found that maize has 35 additional microRNA (miRNA) genes compared to sorghum, almost all directly attributable to duplication and retention from polyploidy. Together, these results suggest that polyploidization contributes to the evolution of regulatory network complexity. Future challenges include experiments to understand how duplicate genes may have diverged in function. Classical forward genetics, large-scale expression studies, and analysis of the maize epigenome may reveal additional patterns of bias between the two subgenomes of maize.

Surveying the Genetic Diversity in Crops Using High-throughput Sequencing Platforms

Having a comprehensive catalog of genetic variation in a species paves the way toward genome-wide genotype-to-phenotype association studies. In agricultural plant species, this will help with the identification of genetic loci underlying important agronomic traits such as yield, disease resistance, and agricultural sustainability. During the past year, our group has been at the forefront of a wave of studies leveraging advances in DNA-sequencing technologies to assess genetic variation. We have developed a robust bioinformatics platform for identifying DNA variation from billions of short DNA sequences and utilized it to create high-resolution variation maps of two important agricultural crops: maize and grapevine.

Constructing a haplotype map of maize. Our collaborators at Cornell University, the University of Missouri, and the North Carolina State University recently constructed the maize nested association mapping (NAM) population, consisting of 27 diverse varieties. This NAM population represents much of the genetic and phenotypic diversity in maize and is a significant resource for phenotypic trait analysis in this crop. Because an overrepresentation of highly repetitive DNA elements exists in the maize genome, we used a strategy that selectively sampled the gene space of the genome, and utilized Illumina sequencing technology to produce more than 32 billion DNA base pairs across the 27 varieties. From these reads, we identified more than 3.3 million single-nucleotide polymorphisms (SNPs) in the genome. The genotypes and distribution of these SNPs are also valuable in estimating population genetics parameters in the maize (Fig. 2).

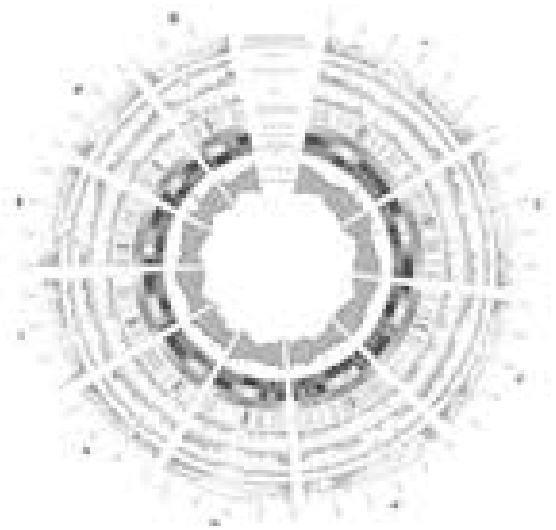

Figure 2. Diversity and recombination across the 10 maize chromosomes. Concentric plots, from inner to outer rings: SNP distribution, resequencing coverage, population recombination rate, gene density, repeat density, karotypes, FST, nucleotide diversity, Tajima's D, recombination rate versus heterozygosity.

From these SNPs, we were able to infer that the genetic diversity among these maize varieties averages one difference every 44 bp, and we believe that this is a lower boundary of the estimated diversity. We also found that an average of 7% of DNA sequences from any other maize variety cannot be mapped to the reference genome, indicating that there is a certain amount of unique genome space in each variety, marked by larger structural DNA variation or peppered with many SNPs.

We also compared the empirically determined population recombination rate of the NAM population with the estimated historical recombination rate and found them to be highly correlated. This suggests that there still is retention of factors that influence recombination, which has not evolved much over time. We also found a significant inverse correlation between recombination and residual heterozygosity in these inbred maize lines, with residual heterozygosity more prevalent in the pericentromeric regions. This suggests that the phenomenon of hybrid vigor, where the progeny of two genetically different parents has superior traits to either parent, may be a result of genes within these pericentromeric regions. In other words, formation of recombinant haplotypes with favorable allelic combinations are restricted by the lack of recombination, supporting the theory that pseudo-overdominance accounts for hybrid vigor in F_1 hybrids. We could also identify large genomic segments with a high recombination rate but low nucleotide diversity; these regions are candidate regions for domestication loci in the maize genome, and 37 of these have lower diversity than the known domestication gene *tb1*.

Given that maize is cultivated across various climates, we wanted to see if we could differentiate tropical and temperate varieties of maize based on their genetic differences. Using a principal component analysis, we could identify dimensions that would sufficiently separate out the tropical, temperate, and sweet corn varieties.

We believe that this haplotype map will be a valuable resource for genetic mapping studies in this NAM population, and we are expecting exciting results from this in the near future.

Genetic diversity of the grapevine. Large, genome-wide genetic mapping studies for important agronomic trait loci in the grapevine have been hamstrung by the absence of a high-resolution variation and linkage-disequilibrium map of the genome. In this study, we leveraged the advances in DNA sequencing technologies to assess the natural DNA variation that exists in this important fruit crop.

A sample of 10 grape cultivars and seven closely related wild-grape species were sequenced. Using a series of heuristic filters that separate genuine polymorphisms from variant repetitive elements and sequencing errors, we are able to derive a set of more than 470,000 markers that segregate in the grapevine. Of these, more than 71,000 scored with high confidence. Besides being a resource for enabling genome-wide association studies, these markers allowed us to survey the linkage-disequilibrium (LD) structure of the grapevine. We found that LD decays rapidly across the genome, and consequently, a high density of markers will be needed to perform an effective association study.

A genotyping microarray was also designed by carefully selecting 9000 markers that were informative in separating the cultivars and wild species. Using this Vitis9KSNP chip to genotype a set of grapevine samples and doing a principal component analysis, we showed that these markers are sufficient in distinguishing between grapevine cultivars, hybrids, and wild species (Fig. 3). We are currently using this array to genotype more than 1200 cultivated grape and 250 wild-grape species that are part of the USDA germplasm repository.

The results from this work suggest that genotyping microarrays are useful for assessing population structure, verifying germplasm collections, and for breeders to corroborate their stock. However, genome-wide genetic mapping studies in high-diversity plant species such as the grapevine are better served using high-throughput

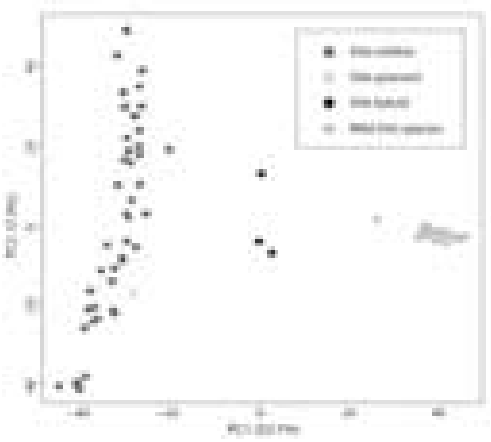

Figure 3. Discriminating grape varieties based on genotypes assayed using the 10K chip array. A principal component analysis shows that the genotypes from the 10K chip array is sufficient to group grape varieties into phylogenetic classifications. This chip is now being used to genotype the USDA grape germplasm collection.

sequencing platforms for genotyping. These sequencing technologies provide the necessary resolution for sufficiently powered association studies that are otherwise not cost effective using genotyping arrays.

Characterizing Regulatory Networks in Plants

The advances in various experimental and bioinformatics techniques in the last decade have provided notable impetus to our knowledge of gene regulatory mechanisms. To develop a systems-level understanding of regulatory networks, we are focused on the interplay of transcriptional and posttranscriptional regulatory mechanisms that operate under abiotic stress conditions and during reproductive development. The regulatory machinery associated with development and plant responses to the environment uses two key control mechanisms: one at the transcriptional level and the other at the posttranscriptional level. The molecular basis for transcriptional regulation is the binding of transcription factors (TFs) to transcription-factor-binding sites and the impact of these interactions to activate or repress gene expression. At the posttranscriptional level, miRNAs regulate gene expression through cleavage of mRNAs or repression of protein production. miRNAs are small noncoding RNAs that regulate many genes involved in developmental control, developmental transitions, plant growth, development, and stress response.

miRNA gene discovery and target characterization. We conducted a genome-wide survey of maize miRNA genes on the first complete assembly of the maize B73 reference genome version 1(B73 RefGen_v1). We developed stringent and robust computational pipelines to predict miRNAs and their targets. On the basis of computational approaches using homology and secondary structure modeling, we identified 150 high-confidence genes within 26 miRNA families. Of these 26 miRNA families, the expressions of 25 were verified in five maize tissue types (root, seedling, tassel, ear, and pollen) by deep sequencing of small RNA libraries. Polymerase chain reaction–rapid amplification of cDNA ends (PCR-RACE) of 68 miRNA transcript precursors, representing 18 families conserved across several plant species, showed that splice variation and the use of alternative transcriptional start and stop sites are common within this class of genes.

Our computational target prediction pipeline identified 247 unique putative targets of these miRNA families. We also predicted putative targets of five miRNA families (miR482, miR528, miR529, miR827, and miR1432) not previously identified in maize. More than 50% of miRNA families were predicted to target TFs, suggesting a predominant role of these miRNA genes in the regulation of transcriptional networks. Characterization of the functions of the target genes of all 26 miRNA families, on the basis of gene ontology, supported their roles in a wide spectrum of regulatory functions and biological processes including gene expression, metabolism, catalysis, transport, and response to stimuli.

Yeast one-hybrid is a gene-centric approach for mapping regulatory relationships. We are using the yeast one-hybrid (Y1H) system to identify transcription factors that bind to specific gene promoters. In the last year, we screened 171 *Arabidopsis* TFs expressed in root stele against 28 miRNA promoterss. We observed 20 interactions out of 4104 possible interactions between 15 transcription factors and eight miRNA promoters. This information will help advance our knowledge of regulatory networks associated with development and crop stress tolerance and provide direction for crop improvement. This work is a collaboration with Drs. Philip Benfey (Duke, Marian Walhout of University of Massachusetts) and Siobhan Brady (University of California, Davis).

Using ultra-high-throughput sequencing to characterize expression profiles of mRNA and small RNAs. Genome-wide expression signatures that mark specific perturbations in developmental programs contribute to functional resolution of central regulators. In this work, we are using ultra-high-throughput sequencing approaches to profile genome-wide transcriptional changes in maize (*Zea mays* L.) reproductive tissues at specific stages of development. We also use mutations in key developmental genes to test the effect of gene-specific perturbations in normal reproductive growth at the transcriptional level. Genetic studies have previously anchored these genes into core developmental pathways. Here, computational methods are used to integrate and analyze expression data sets, which will provide a basis for inferring the gene networks associated with these pathways. This work is a joint project with David Jackson at CSHL.

With the recent release of an assembled maize genome sequence, we use this resource to build robust and well-documented pipelines for mapping and analysis of high-throughput, sequence-based transcript data in maize. Next-generation sequencing approaches currently being used include tag-based digital gene expres-

sion (DGE) and RNA-Seq. Our evaluation of these methods in association with the maize genome compares throughput, specificity, and coverage of expressed genes, including resolution of quantitative expression profiles for low-copy transcripts. Various statistical methods are also being evaluated for analysis of differentially expressed genes. On the basis of these analyses, standardized methods are being developed for incorporating new data sets as they become available.

Early stages of maize ear and tassel development, which are the female and male reproductive organs, respectively, are investigated in this work. By profiling normal development in these tissues using a whole-transcriptome RNA-Seq approach, we can improve current maize gene models and use the transcript profiles to anchor expression data from mutant ears and tassels at the same stage of development. Groups of genes showing differential expression or coexpression in response to specific perturbations are identified. Comparative genomics and bioinformatics tools are used to leverage existing information from *Arabidopsis* and rice to orthologous maize genes for which functional annotations are not known. On the basis of such comparative approaches, genes in common biochemical pathways are identified using well-curated pathway tools in related species. These data provide curation of metabolic pathways in maize. We also use sequence similarities to identify transcriptional regulators in maize. Integration of data sets from mutant-based perturbations will help determine which of these transcription factors are central components in the transcriptional network and thus candidates for downstream functional and genetic experiments. These analyses will provide evidence for regulators of maize ear and tassel architecture, an important agricultural trait with clear relevance to grain yield and harvestability.

Gramene Comparative Genomics: A Model for Understanding Function through Evolution and Genome Organization

The ability to connect information within and across species through comparative mapping, integration of genomic sequence, genetic and physical maps, and phenotypes adds significant value to existing genome-sequencing and genome-mapping studies. Gramene is a collaborative project that leverages sequence and functional information from multiple plant genomes to promote translational genomics in agriculture. The Gramene website (http://www.gramene.org) serves as a portal to multiple genome browsers and manually curated databases of genes, proteins, biochemical pathways, quantitative trait loci (QTL), ontologies, germplasm, and genetic diversity data.

In the last year, our group has completed several milestones on the Gramene project, including the release of the 29th and 30th builds since 2000. Of the many improvements made, we increased the number of sequenced genomes in our Ensembl genome browser to 13 agronomically and scientifically important species. These databases were created in partnership with the European Bioinformatics Institute (EBI) and are now also available through the Ensembl Plant portal. The collaboration with EBI has focused on the integration of content, quality control, and the development of new features. A major addition in the last year has been the new large-scale SNP-chip-based genotype data sets for rice and *Arabidopsis*, plus the addition of a basic coordinate-based search tool that allows users to retrieve allele calls. Finally, Gramene added an entirely new module focusing on germplasm resources targeted at the breeding community.

The iPlant Collaborative

The iPlant Collaborative (http://iplantcollaborative.org) is an NSF-funded cyberinfrastructure collaborative for the plant sciences. Its mission is to foster the development of a diverse, multidisciplinary community of scientists, teachers, and students and a cyberinfrastructure that facilitates significant advances in the understanding of plant science through the application of computational thinking and approaches to Grand Challenge problems in plant biology.

Currently there are two community-defined Grand Challenge projects: (1)Tree of life (iPToL) and (2) genotype to phenotype (iPG2P). The iPToL project seeks to resolve the evolutionary relationships among the half-million known species of green plants and to convey these in the form of comprehensive phylogenetic trees. Owing to its sheer scale, complexity, and profound nature, this undertaking represents a true "moonshot" for the life sciences. The required cyberinfrastructure will scale up current phylogenetic methods by 100-fold or more, enable the dissemination of data associated with such large trees, and implement scalable "post-tree" analysis tools to foster integration of the plant tree of life with the rest of the botanical sciences. The iPG2P project aims to elucidate the relationships between plant genotypes and the resultant phenotypes in complex environments, one of the foremost challenges in biology.

This year's effort was directed at tool and method development to analyze and integrate next-generation sequence data and permit broader access of these resources to the general research community. Broader impacts of the iPlant Collaborative will be achieved not only by solving Grand Challenge questions, but also by its focus on community building and educational outreach. The DNA Subway project is a collaboration between the iPlant Collaborative and the Dolan DNA Learning Center to provide high-level genome analysis tools to biology teachers and students. Several members of the Ware lab actively participate in working groups of the iPToL and iPG2P Grand Challenge projects. Through requirements analysis, project management and coordination, prototyping, and direct engagement, they facilitate communication between the community collaborators and the cyberinfrastucture developers.

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COMPUTATIONAL GENOMICS

M.Q. Zhang M. Akerman, X. Chen, W. Chung, M. Kato, J. Kinney, D. Lewis, W. Liao, W. Luo, Y. Ma, A. Mail, M. Oswald-Pisarski, M. Paradesi, M. Tang, K. Wrzeszczynski, J. Wu, Z. Xuan, C. Xue, Z. Zhang

In the last year, New York University student Jeff Rosenfeld finished his Ph.D. and took a staff scientist position at Long Island Jewish Hospital. Xiaowo Wang took an assistant professor position at Tsinghua University in China, Dr. Michaela Oswald-Pisarski moved to the Wigler lab, and Dr. Chenghai Xue moved to the Gingeras lab here at CSHL. Avila Mail was our URP student from Cornell University and Xuxin Chen was our rotation student from Stony Brook University. Dr. Zhenyu Xuan has started a new tenure-track faculty position at University of Texas, Dallas. I have accepted an endowed Cecil H. and Ida Green Distinguished Chair Professor and a directorship of a new Center for Systems Biology at the University of Texas, Dallas. Two postdoctoral fellows, Drs. Zhihua Zhang and Wemyu Chung, have also moved to the new lab at the University of Texas, Dallas.

High-resolution Human Core Promoter Prediction with CoreBoost_HM

Correctly locating the gene transcription start site and the core promoter is important for understanding the transcriptional regulation mechanism. We have integrated specific genome-wide histone modification and DNA sequence features together to predict RNA polymerase II core promoters in the human genome. Our new predictor *CoreBoost_HM*, which is an extension of our previous sequence-based algorithm *CoreBoost*, outperforms all existing promoter prediction algorithms by providing significantly higher sensitivity and specificity at high resolution. We demonstrated that even though the histone modification data used in this study are from a specific cell type ($CD4^+$ T cell), our method can be used to identify both active and repressed promoters. We have applied it to search the upstream regions of microRNA (miRNA) genes and show that CoreBoost_HM can accurately identify the known promoters of the intergenic miRNAs. We also identified a few intronic miRNAs that may have their own promoters. This result suggests that our new method can help to identify and characterize the core promoters of both coding and noncoding genes (Wang et al. 2009).

Determination of Enriched Histone Modifications in Nongenic Portions of the Human Genome

Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) has recently been used to identify the modification patterns for the methylation and acetylation of many different histone tails in genes and enhancers. In collaboration with the Zhao lab at the National Institutes of Health, we have extended our previous analysis of histone modifications (Wang et al., *Nat Genet 40:* 897 [2008]) to gene deserts, pericentromeres, and subtelomeres. Using data from human $CD4^+$ T cells, we have found that each of these nongenic regions has a particular profile of histone modifications that distinguish it from the other noncoding regions. Different methylation states of H4K20, H3K9, and H3K27 were found to be enriched in each region relative to the other regions. These findings indicate that nongenic regions of the genome are variable with respect to histone modification patterns, rather than being monolithic. We furthermore used consensus sequences for unassembled centromeres and telomeres to identify the significant histone modifications in these regions. Finally, we compared the modification patterns in nongenic regions to those at silent genes and genes with higher levels of expression. For all tested methylations with the exception of H3K27me3, the enrichment level of each modification state for silent genes is between that of nongenic regions and expressed genes. For H3K27me3, the highest levels are found in silent genes. In addition to the histone modification pattern difference between euchromatin and heterochromatin regions, as is illustrated by the enrichment of H3K9me2/3 in nongenic regions, while H3K9me1 is enriched at active genes, the chromatin modifications within nongenic (heterochromatin-like) regions (e.g., subtelomeres, pericentromeres, and gene deserts) are also quite different. (Rosenfeld et al. 2009).

An Integrative Genomics Approach Identifies Hypoxia-inducible Factor 1 Target Genes that Form the Core Response to Hypoxia

The transcription factor hypoxia-inducible factor 1 (HIF-1) has a central role in the transcriptional response to oxygen flux. To gain insight into the molecular pathways regulated by HIF-1, it is essential to identify the downstream target genes. In collaboration with the Xavier lab at Massachusetts General Hospital/Harvard Medical School, we developed a strategy to identify HIF-1-target genes based on an integrative genomic approach combining computational strategies and experimental validation. To identify HIF-1 target genes, microarrays data sets were used to rank genes based on their differential response to hypoxia. The proximal promoters of these genes were then analyzed for the presence of conserved HIF-1-binding sites. Genes were scored and ranked based on their response to hypoxia and their HIF-binding site score. Using this strategy, we recovered 41% of the previously confirmed HIF-1 target genes that responded to hypoxia in the microarrays; we also produced a catalog of predicted HIF-1 targets. In addition, we carried out experimental validation for ANKRD37 as a novel HIF-1 target gene. Together, these analyses demonstrate the potential to recover novel HIF-1 target genes and the discovery of mammalian regulatory elements operative in the context of microarray data sets (Benita et al. 2009).

High-definition Profiling of Mammalian DNA Methylation by Array Capture and Single-molecule Bisulfite Sequencing

DNA methylation stabilizes developmentally programmed gene expression states. Aberrant methylation is associated with disease progression and is a common feature of cancer genomes. Presently, few methods enable quantitative, large-scale, single-base resolution mapping of DNA methylation states in desired regions of a complex mammalian genome. In collaboration with the Hicks, Hannon, Wigler, and McCombie labs at CSHL, we developed an approach that combines array-based hybrid selection and massively parallel bisulfite sequencing to profile DNA methylation in genomic regions spanning hundreds of thousands of bases. This single-molecule strategy enables methylation-variable positions to be quantitatively examined with high sampling precision. Using bisulfite capture, we assessed methylation patterns across 324 randomly selected CpG islands (CGI), representing more than 25,000 CpG sites. A single lane of Illumina sequencing permitted methylation states to be definitively called for more than 90% of target sites. The accuracy of the hybrid selection approach was verified using conventional bisulfite capillary sequencing of cloned polymerase chain reaction (PCR) products amplified from a subset of the selected regions. This confirmed that even partially methylated states could be successfully called. A comparison of human primary and cancer cells revealed multiple differentially methylated regions. More than 25% of islands showed complex methylation patterns either with partial methylation states defining the entire CGI or with contrasting methylation states appearing in specific regional blocks within the island. We observed that transitions in methylation state often correlate with genomic landmarks, including transcriptional start sites and intron–exon junctions. Methylation, along with specific histone marks, was enriched in exonic regions, suggesting that chromatin states can foreshadow the content of mature mRNAs. (Hodges et al. 2009).

To prepare the computational algorithm for this work, we have extended our previous Solexa reads-mapping algorithm *RMAP* to allow mapping of bisulfite sequencing reads. We have also added the capability of analyzing pair-end reads as well as single reads (Smith et al. 2009). This new algorithm has been very popular and downloaded by many labs around the world.

The Transcriptome of Human CD34$^+$ Hematopoietic Stem-progenitor Cells

Studying gene expression at different hematopoietic stages provides insight for understanding the genetic basis of hematopoiesis. In collaboration with the Wang lab at Northwestern University and the Rowley lab at Chicago University, we analyzed gene expression in human CD34$^+$ hematopoietic cells that represent the stem-progenitor population (CD34$^+$ cells). We collected more than 459,000 transcript signatures from CD34$^+$ cells, including the de-novo-generated 3′ expressed sequence tags (3′ ESTs) and the existing sequences of full-length cDNAs, ESTs, and serial analysis of gene expression (SAGE) tags, and performed an extensive annotation on this large set of CD34$^+$ transcript sequences. We determined the genes expressed in CD34$^+$ cells, verified the known genes, and identified the new genes of different functional categories involved in hematopoiesis; dissected alternative gene expression including alternative transcription initiation,

splicing, and adenylation; identified the antisense and noncoding transcripts; determined the $CD34^+$ cell-specific gene expression signature; and developed the $CD34^+$ cell transcription map in the human genome. Our study provides a current view on gene expression in human $CD34^+$ cells and reveals that early hematopoiesis is an orchestrated process with the involvement of more than half of the human genes distributed in various functions. The data generated from our study provide a comprehensive and uniform resource for studying hematopoiesis and stem cell biology (Kim et al. 2009).

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Kazimierz Wrzeszczynski (*left*) and Michael Zhang

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, most notably, Carol Greider, last year's recipient of the Nobel Price in Physiology or Medicine for her work on telomerase and telomere function. She joined the Fellows program in 1998 and, after completing her fellowship, was a member of the CSHL faculty for 9 years. She is currently the Daniel Nathans Professor and Director of Molecular Biology and Genetics at Johns Hopkins University School of Medicine.

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. Eric Richards (1989) is currently the Vice President of Research and a scientist at the Boyce Thompson Institute for Plant Research at Cornell University, and David Barford (1991) is a Fellow of the Royal Society and a 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, University of Zürich, Switzerland. Térence Strick (2000) left at the end of his fellowship to become a Group Leader at the Institute Jacques Monod, Centre National de la Recherche Scientifique and Universités de Paris VI and VII. Both Lee Henry (2000) and Ira Hall (2004) moved to Virginia upon completion of their fellowships. Lee joined a project headed by Thomas Südhof at HHMI's Janelia Farm in Ashburn, and Ira is Assistant Professor in the Department of Biochemistry and Molecular Genetics at the University of Virginia in Charlottesville. Patrick Paddison, who had joined the Fellows Program in 2004, recently left to become an Assistant Member at the Fred Hutchinson Cancer Research Center in Seattle, Washington.

Currently, the lab hosts two Fellows: 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 optogenetic 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, where he studied chromatin looping mechanisms in long-range enhancer function. As a Fellow, Chris is studying the role of histone lysine methylation in normal and malignant hematopoiesis.

Understanding Neuronal Circuits in the Mammalian Olfactory Bulb

A. Dhawale, M. Davis, F. Albeanu

In many regions of the brain, neurons form an ordered representation of the outside world. For example, the "homunculus" of the somatosensory cortex is a point-to-point topographic map of the body surface onto the brain surface. The spatially organized convergence of sensory inputs often leads to similar response properties in target neurons that are in the close vicinity. Whether their individual information content is redundant or independent depends on the circuit architecture (the interplay between common input, lateral signals, and feedback from other brain areas) and the computational goals of the network.

We use optogenetic tools (such as two-photon imaging of genetically encoded neuronal activity reporters or light-gated neuronal activity switches) coupled with electrophysiological measurements (extracellular and intracellular recordings) to study how sensory systems

encode information from the environment. We want to understand how these inputs get processed at different junctions or synapses of the underlying neuronal circuits and how these representations change with the state of the system and its circuits (awake vs. anesthetized, naïve vs. conditioned). The broad scope of this effort is observing how perceptions arise.

The olfactory system, particularly the olfactory bulb (OB), in rodents provides us with an ideal substrate to answer these questions, given its well-defined circuitry and multilayered organization. Rodents, being nocturnal animals, depend heavily on olfaction for survival—finding food, mates or avoiding predators. Airborne chemicals or odors are translated into neuronal signals by specific receptors in the nose and sent first to the bulb and then to higher centers in the brain (olfactory cortex). The bulb thus being the relay center provides the opportunity to study both the nature of the inputs it receives and the nature of output it sends to the brain and the computations that allow for this input–output transformation.

In the bulb, sensory neurons expressing the same type of olfactory receptor converge in tight focus, forming ~2000 clusters of synapses called glomeruli. The layout of glomeruli on the bulb is highly reproducible across individuals with a precision of one part in 1000. However, nearby glomeruli are as diverse in their responses to odors as distant ones, lacking an apparent chemotopic arrangement (Soucy et al. 2009). From each glomerulus, a few dozen mitral cells (principal output neurons of the OB) carry the output further to the olfactory cortex and several other brain areas. The mitral cells typically have only one primary dendrite that projects to a single glomerulus, but they can sample inputs on their primary and secondary dendrites from functionally diverse glomeruli via several types of interneurons (Fig. 1). Thus, a few dozen mitral cells share input from the same parent glomerulus (sister cells), but they may have different inhibitory surrounds.

Are Outputs from the Same Glomerulus Redundant?

We generated transgenic mice expressing channelrhodopsin-2 in all olfactory sensory neurons under the control of the olfactory marker protein (OMP) promoter. By selectively stimulating individual glomeruli via DLP patterned illumination, and recording extracellularly from the mitral cell layer using tetrodes, we identified mitral cells that receive common input (Fig. 2). We found that sister mitral cells had highly correlated responses to odors as measured by average spike rates, but their spike timing with respect to respiration was differentially altered. In contrast, nonsister mitral cells correlated poorly on both these measures. We therefore propose that sister cells carry two different channels of information to the cortex: (1) average activity representing shared glomerular input and (2) phase-specific information that refines odor representations and is substantially independent for sister mitral cells.

What Roles Does the Inhibitory Network Have in Shaping the Bulb Outputs?

Activity in the bulb is a rich mix of excitation and inhibition, via both direct inputs and feedforward and feedback connections by a wide variety of cell types. Juxta-glomerular cells are local interneurons that surround the glomeruli and send neurites within and across glomeruli. In the external plexiform layer, another class

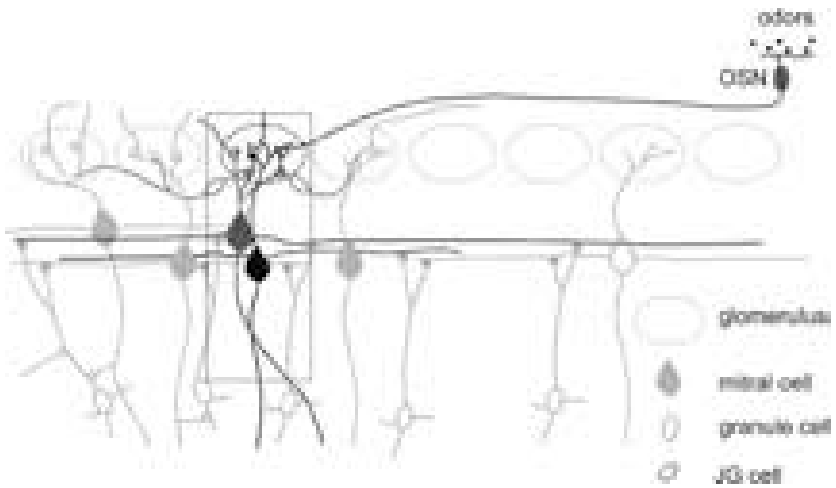

Figure 1. Main olfactory bulb wiring schematics. (Courtesy of F. Markopoulos.)

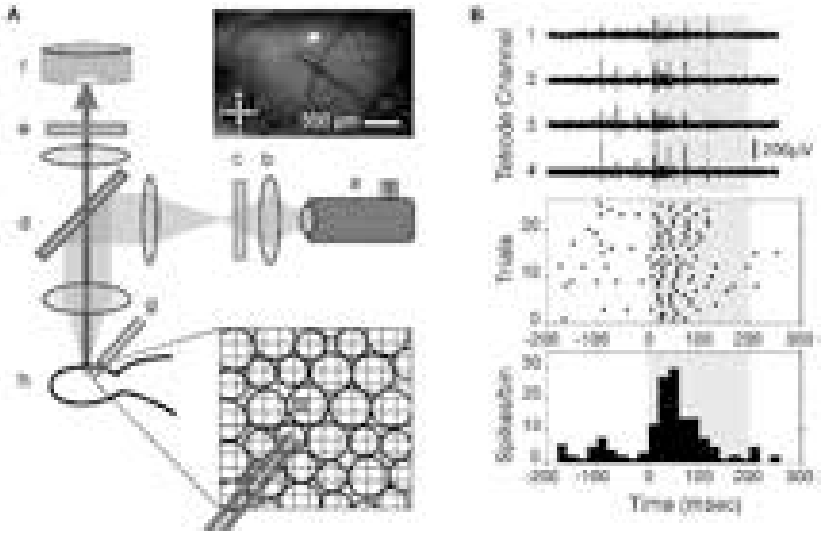

Figure 2. DLP patterned illumination in ORC mice maps the parent glomeruli of mitral cells in vivo. (*A, Left*) A DLP projector coupled to a system of three lenses delivered light patterns to activate individual glomeruli on the dorsal surface of the bulb. A CCD (charge-coupled device) camera was used to visualize the bulb surface and record the position of the light stimulus. (*Top panel, right*) Dorsal surface of the bulb with a tetrode positioned in the mitral cell layer. One square light spot is being projected onto the surface of the bulb. (*Inset*) Cartoon schematic of glomeruli on the bulb, a subglomerular-size light spot and dual tetrodes positioned in the mitral cell layer. (*a*) DLP projector, with color wheel removed from optical path, (*b*) focusing lens, (*c*) blue excitation filter, (*d*) dichroic mirror, (*e*) emission filter, (*f*) CCD camera, (*g*) dual-tetrode, (*h*) olfactory bulb. (*B, Top*) Raw voltage traces corresponding to the four channels of a tetrode showing light-induced changes in firing patterns of the units recorded. (Center) Raster plot shows changes in spiking activity of a single isolated unit across 25 light-stimulation trials, each 200 msec long; dots mark spike occurrence times. (*Bottom*) Peristimulus time histogram (PSTH) with 25-msec time bins, summing spikes over the trials shown in the center panel.

of interneurons, the granule cells, mediates cross-talk between mitral cells via reciprocal synapses with lateral mitral cell dendrites (Fig. 1). Both interneuron types are generic denominations for heterogeneous neuronal populations with respect to wiring, gene expression, and physiological properties. Despite decades of study in the slice preparation, little is known about how these interneurons operate in the intact circuit in vivo.

First, we aimed to understand if bulb interneurons modulate the spiking time of mitral cells. We have started by expressing light-gated molecular switches of neuronal activity (such as channelrhodopsin-2 and halorhodopsin) in genetically defined subsets of bulb interneurons. We are using a Cre/Lox strategy and taking advantage of several interneuron-specific Cre lines generated by Josh Huang' group at CSHL.

Optical Dissection of Olfactory Microcircuits: What Computations are Performed on the Inputs by the Bulb Circuitry?

We set out to sample the activity of large and diverse neuronal populations from the two layers of the bulb in response to numerous odorants. Recent developments in multiphoton microscopy and new generations of genetically encoded neuronal activity reporters allow monitoring both glomeruli (inputs) and mitral cells (outputs) responses simultaneously. Toward this end, we built two custom multiphoton microscopes and two large-scale odor delivery machines (165 odorants). Preliminary experiments indicate that the activity patterns at the level of mitral cell bodies are not simply scaled down versions of glomerular representations (Fig. 3). Therefore, we have started to systematically investigate the contributions of lateral signals and feedback from other areas to bulb input-output transform via optogenetic strategies.

The Bulb Vision Project

We are using light activation of glomeruli in awake, head-restrained OMP-chr2 mice to ask how different spatiotemporal input patterns get processed at the level of the mitral cells and influence behavior. Using light activation of the glomeruli bypasses the inherent uncertainty of olfactory stimuli delivery, as odor plumes diffuse slowly and in complex patterns. Our goal is to generate olfactory percepts by strictly controlled pat-

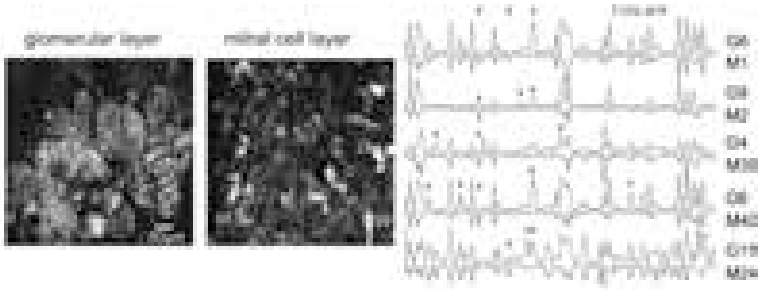

Figure 3. Odor responses of glomerular and mitral cell body layers via multiphoton imaging of GCAMP2 signals. (*Left*) Glomeruli, (*center*) mitral cell bodies, (*right*) best functional matches between glomerular and mitral cell body odor responses to a panel of 125 stimuli. (G) Glomerulus, (M) mitral cells from Kv3.1 GCAMP2 mice. (Fletcher et al., *J Neurophysiol 102:* 817–830 [2009].)

terned light stimulation and dissect the neurocorrelates of these patterns that dictate olfactory behavior at the level of olfactory bulb circuitry.

Other Collaborative Projects with CSHL Groups

We are also collaborating with fellow scientists on the following projects: Kepecs: optical monitoring of cholinergic modulation of cortical circuits; Koulakov and Hannon: bringing the gap between the functional responses of glomeruli to odors and odor receptor sequences; Li and Shea: monitoring synaptic plasticity of bulb circuits and AMPA receptor trafficking in real time, during learning; Zador: optical monitoring of neuronal activity in genetically and anatomically defined cortical circuits in animal models of cognition; and Jackson: using optical tools to study molecular mechanisms controlling branching of maize inflorescence.

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Epigenetic Inheritance and Cancer

C.R. Vakoc

Chromatin is the fundamental packaging material of eukaryotic genomes, composed of DNA in its native complex with protein. The basic organizational unit of chromatin is the nucleosome, composed of an octamer of core histones wrapped by 147 bp of DNA. Chromatin is charged with the critically important task of both protecting the genetic code and animating its many functions. Replication, recombination, and transcription of DNA in the cell all occur in a chromatin context, and hence, elaborate control mechanisms have evolved to govern chromatin structure in these diverse scenarios. The central interest of our laboratory is to understand the enzymatic mechanisms that control chromatin and how such pathways establish heritable chromatin states and participate in the pathophysiology of cancer.

A major focus of our work is a class of molecules referred to as human Trithorax proteins, or also known as the mixed-lineage leukemia (MLL) family. MLL proteins are large modular chromatin regulators that utilize catalytic (methyltransferase activity of histone H3 lysine 4) and noncatalytic mechanisms (chromatin-binding and scaffold functions) to maintain the "ON" state of gene expression. One fascinating feature of MLL orthologs in other species is their unique capacity to maintain heritable states of gene activity, as exemplified by the MLL orthologs in *Drosophila* maintaining heritable programs of *Hox* gene expression, a phenomenon known as cellular memory. Although most of the classic epigenetic inheritance phenomena have been classically linked with the "OFF" state of expression, the epigenetic maintenance of the "ON" state by MLL stands alone as one of the only known mechanisms in nature that stably preserves active chromatin in a manner heritable through somatic cell divisions. A key interest in our laboratory is define the molecular mechanism of epigenetic inheritance by MLL proteins.

In addition to having essential functions in maintaining active chromatin during normal ontogeny and differentiation, the MLL1 protein is also a proto-oncogene in acute leukemia that can be mutated via chromosomal translocation to generate oncogenic fusion proteins. MLL1 fusion proteins induce a hyperactive chromatin state as compared to wild-type MLL1, which leads to up-regulation of MLL1's normal target genes to block hematopoietic cell differentiation and induce leukemia. Thus, MLL1 leukemias represent a clear situation where chromatin dysfunction directly causes a human cancer. In collaboration with Johannes Zuber in Scott Lowe's laboratory at CSHL, we have begun using a mouse model of MLL1 leukemia to explore the causal relationship between chromatin regulation and the pathogenesis of cancer.

Epigenetic Inheritance Mechanisms Used by MLL1

Evidence across many metazoan species implicates the MLL1 protein in preserving a mitotically heritable "ON" chromatin state. However, the molecular mechanisms underlying this function have not been determined. MLL1 possesses a catalytic SET domain that labels histone molecules in the nucleosome with H3K4 methylation. Although plenty of evidence suggests that histone methylation can participate in maintaining heritable chromatin states in various contexts, the SET domain of MLL is actually not required to perform most of its essential functions in vivo, i.e., an MLL-null mouse is embryonic lethal, whereas MLLΔSET mice are born at normal mendelian ratios with a minimal phenotype (Terranova et al., *Proc Natl Acad Sci 103:* 6629–6634 [2006]). This observation suggests that MLL must use an additional noncatalytic means of maintaining heritable active chromatin. With this question in mind, we set out to investigate whether MLL has evolved specialized mechanisms to protect active chromatin from disruption during cell division which might underlie an inheritance function. We chose to investigate MLL's status during M phase of the cell cycle, because during mitosis, gene activity is temporarily extinguished and must self-renew within daughter cell chromatin upon re-entry into G_1. Indeed, the condensed state of mitotic chromosomes basically prevents access of most chromatin-associated proteins during M phase. To our surprise, however, we found that MLL remains attached to condensed mitotic chromosomes as revealed by immunofluorescence in several cell types. We then used genome-wide location analysis to identify the mitotic target genes of MLL, which revealed that MLL has essentially latched on to the genes that were the most highly expressed in the genome, prior to the cells having entered mitosis and silenced gene transcription. This observation suggested to us that MLL might be "bookmarking" gene activity during mitosis, wherein MLL itself might be serving as an epigenetic mark to tell daughter cells which genes to express upon completion of mitosis. Consistent with this hypothesis, we found that when MLL was depleted, daughter cells displayed delayed kinetics of gene reactivation. We went on to show that MLL tethers additional chromatin regulators (Menin, RbBP5, and Ash2L) to its occupied sites within mitotic chromatin to "mark" sites of premitotic gene activity.

Collectively, our observations support the "MLL epigenetic mark" as a widespread mechanism to faithfully and efficiently restore gene activity following mitosis in human cells. We speculate that the ultimate service this mechanism provides to cellular tissues is to supply momentum to the status quo and allow dividing cells to resist changes in gene expression. This would be supported by MLL knockout phenotypes in vivo, where hematopoietic cells exhibit compromised self-renewal and instead aberrantly differentiate and adopt more-differentiated cell fates. In follow-up to this work, we seek to identify the precise molecular mechanism by

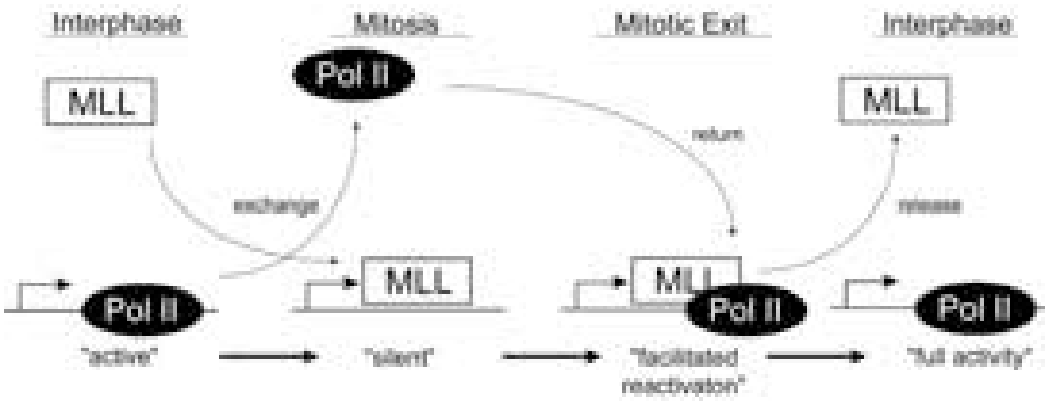

Figure 4. The MLL1 protein as an epigenetic mark that facilitates inheritance of gene expression states through mitosis. The transition from G_2 into M phase is associated with eviction of RNA polymerase II (pol II) and most DNA-binding proteins from their interphase chromosomal locations. The exodus of transcription machinery is coupled with the acquisition of MLL1 occupancy to "mark" locations that possessed premitotic gene activity. MLL1 occupancy endures through chromosome inheritance to each daughter cell. Upon completion of mitosis, MLL facilitates the return of transcriptional machinery back to its premitotic locations, likely through direct physical interactions with the largest subunit of RNA pol II. Upon restoration of high-level transcription, MLL is redistributed away from its mitotic locations to novel interphase locations where it serves as a coactivator for a number of DNA-binding transcription factors.

which MLL uniquely attaches itself to mitotic chromatin. In addition, a continued interest of ours is in evaluating how progression through mitosis brings about the capacity for changes in gene expression states, and how mechanisms like that described above provide resistance to reprogramming cellular state.

MLL Leukemia: A Model to Study the Causality between Chromatin Malfunction and Cancer

In addition to the unique molecular features of wild-type MLL, mutant forms of MLL found in leukemia also exploit chromatin-regulatory mechanisms to block myeloid cell differentiation and maintain a leukemic phenotype. A major long-term goal of the lab is to use a mouse model of leukemia as a model system to study epigenetic inheritance and to explore the therapeutic potential of manipulating chromatin regulators to reverse the leukemia phenotype. In the past year, we have initiated an exciting collaboration with Johannes Zuber in Scott Lowe's laboratory to investigate how chromatin regulators participate in the pathogenesis of MLL leukemia. Using a combination of in vivo delivery of conditional RNA interference (RNAi) together with biochemical assays of chromatin in leukemic cells, we seek to gain a robust structure–function relationship between mutant forms of MLL that maintain the disease and the epigenetic "lesions" they inflict upon the chromatin fiber.

PUBLICATIONS

Blobel GA, Kadauke S, Wang E, Lau AW, Zuber J, Chou MM, Vakoc CR. 2009. A reconfigured pattern of MLL occupancy within mitotic chromatin promotes rapid transcriptional reactivation following mitotic exit. *Mol Cell* **36:** 970–983.

Chou ST, Khandros E, Bailey LC, Nichols KE, Vakoc CR, Yao Y, Huang Z, Crispino JD, Hardison RC, Blobel GA, et al. 2009. Graded repression of PU.1/Sfpi1 gene transcription by GATA factors regulates hematopoietic cell fate. *Blood* **114:** 983–994.

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Vakoc CR, Wen YW, Johnstone CN, Rustgi AK, Gibbs RA, Blobel GA. 2009. Low frequency of MLL3 mutations in colorectal carcinoma. *Cancer Genet Cytogenet* **189:** 140–141.

Molecular Networks

I. Iossifov

My research is focused on building and refining models of molecular networks, as well as on applying these models to study the genetics of various human diseases. I devise methods to reliably extract knowledge about molecular interactions from the biomedical literature, use data integration methods to combine the literature-based information with results from high-throughput experiments, and build statistical methods to interpret genomic data and to generate hypothesis based on whole-genome molecular networks.

Drosophila Segmentation Network

In collaboration with Dr. Kevin White's lab at the University of Chicago, we built an integrated model of the segmentation network around two key transcription factors (*Ftz* and *Eve*) involved in the process of embryonic segmentation in *Drosophila melanogaster*. In addition to the literature-mined molecular network, the model included lists of differentially expressed genes in *Ftz* and *Eve* null mutants compared to wild type, genes identified through chromatin immunoprecipitation (ChiP)-chip assays for the binding sites of *Ftz* and *Eve*, and a large yeast two-hybrid assay. The fly ortholog of the human uniquitin E3 ligase, *SPOP*, was identified to be central to the integrated model and further analysis revealed its role in modulating tumor necrosis factor (TNF)-mediated JNK (c-Jun N-terminal kinase) signaling and thus its potential role in human cancer. A tissue microarray screen of 18 cancer types showed that *SPOP* was highly expressed in clear-cell renal cell carcinoma (RCC) and that *SPOP*'s expression could be used to distinguish histological subtypes of RCC.

Cerebellum Development through Human and Mouse Networks

We used text mining to extract and purify two large molecular networks, one for humans and one for mice, and used the networks to study the etiology of five cerebellum phenotypes. We demonstrated quantitatively that genes involved in development-related malformations differ in their system-level properties from degeneration-related genes. We showed that there is a high degree of overlap among the genes implicated in developmental malformations, that these genes have a strong tendency to be highly connected within the molecular network, and that they also tend to be clustered together, forming a compact molecular network neighborhood. In contrast, the genes involved in malformations due to degeneration do not have a high degree of connectivity, are not strongly clustered in the network, and do not overlap significantly with the development-related genes. In addition, taking into account the above-mentioned system-level proper-

ties and the gene-specific network interactions, we made highly confident predictions about novel genes that are likely also involved in the etiology of the analyzed phenotypes. Included in our list of predicted genes was the *Ascl1* gene, which is involved in neuronal commitment and differentiation and whose role in cerebellum development was independently demonstrated.

Figure Mining for Biomedical Research

Together with Dr. Raul Rodriguez-Esteban (Pfizer Research Technology Center), we built a search engine to retrieve the figures that are publicly available through PubMed Central. The main feature of our search engine is its ability to search for figures by type in addition to a keyword-based search. For example, one can find all of the pathway diagrams that include p53. We classified all figures into a few high-level classes (gel, pathways, structure, and time) based on the words in the figures' caption, on text within the figure itself (extracted through the use of OCR), and on global image properties such as color histogram and shape structure. We then indexed the figures based on the assigned type, the words in the figures' caption, in the figure itself, and in the article containing the figure.

PUBLICATIONS

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C. Vakoc

AUTHOR INDEX

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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 we are now beginning our second decade and our 11th year!

In April 2009, we welcomed a new member to Team Watson, WSBS Coordinator, Dr. Judith Wieber. Judy has a Ph.D. in biochemistry/physical chemistry and has most recently worked as a program coordinator and instructor in the Department of Computational Biology, School of Medicine, University of Pittsburg. She came to us with a strong background in teaching, course development, publishing, marketing communications, and administration.

Team Watson: (*Left to right*) Leemor Joshua-Tor, Alyson Kass-Eisler, Dawn Pologruto, Kim Geer, and Judy Wieber

Faculty Changes

In 2009, three new faculty members joined the Laboratory: Mikala Egeblad, Alexander Krasnitz, and Stephen Shea. Assistant Professor Mikala Egeblad came to the Watson School from the University of California, San Francisco, where she was a postdoctoral fellow. She studies how the dynamics of the interactions between the tumor microenvironment and cancer cells change during anticancer treatment. Her laboratory uses transgenic mouse models of cancer and a live-imaging method, which she developed, that allows them to watch and quantify the innate immune response to tumors in live mice.

Alexander Krasnitz, an Assistant Professor, first came to CSHL in 2005 as a Senior Computer Scientist in Michael Wigler's laboratory. He is a computational physicist by training with expertise in distilling information from massive quantities of noisy data and interpreting complex experimental outcomes through models. His current research looks at high-level analysis of microarray-derived data in cancer biology.

Assistant Professor Stephen Shea came to the Watson School from Duke University Medical Center, where he was a postdoctoral fellow. The focus of his research is neural circuits and their control of behavior. The long-term goal in his laboratory is to anatomically and physiologically trace complete neural circuits that mediate social decision arcs using communication behavior in mice.

We are very pleased to have each of these exciting newcomers work with our students. They have already become members of student Thesis Committees and Qualifying Exam Committees, and they 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 2009: Jonathan Sebat, Jacek Skowronski, Lincoln Stein, and William Tansey. All four served the School and its students in various roles including instructor, guest lecturer, rotation advisor, mentor, examiner, and Thesis Committee members. Additionally, William Tansey served as the School's Director of Graduate Studies from July 2004 to May 2007 and the Chair of the Curriculum Development and Integration Committee from 2003 to 2007. We are extremely grateful for his contributions to the development of the School's unique and innovative curriculum. We will miss our fellow colleagues, but we wish them well in their new endeavors.

The Sixth WSBS Graduation

Graduation is the highlight of the year and April 26, 2009 saw the Watson School's sixth graduation ceremony. The graduating class of 2009 comprised Allison Blum and Shu-Ling Chiu from the en-

(*Left to right*) WSBS Dean Leemor Joshua-Tor with 2009 graduates Allison Blum, Keisha John, Shu-Ling Chiu, Jeremy Wilusz, Daniel Chitwood, and CSHL President Bruce Stillman

tering class of 2002, Daniel Chitwood and Keisha John from the entering class of 2004, and Jeremy Wilusz from the entering class of 2005, who were awarded the Ph.D. degree. Sarahjane Locke from the entering class of 2005 was awarded a Master's degree. Sarahjane left the program early and is currently working at the University of Vermont as a Research Project Assistant.

This year, the graduation celebrated the educational programs at the Laboratory. Honorary degrees were bestowed on the WSBS founding dean Dr. Winship Herr in recognition of the 10th anniversary of the School, on Dr. Alfred Goldberg in recognition of the 50th anniversary of the Undergraduate Research Program, on Mr. David Micklos in celebration of the 20th year of the Dolan DNA Learning Center, and on Dr. Jeffrey Miller in recognition of his scholarly contributions through the CSHL press. Winship delivered the commencement address recounting all of the excitement in the founding of the School.

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 Taiwan to take part in this special event.

2009 WSBS DOCTORAL RECIPIENTS

Student	Thesis advisor	Academic mentor	Current position
Allison Blum	Josh Dubnau	Hollis Cline	Postdoctoral fellow with Dr. Ruth Lehmann at the Skirball Institute of Biomolecular Medicine, New York University School of Medicine
Daniel H. Chitwood	Marja Timmermans	Alea A. Mills	Postdoctoral fellow with Drs. Neelima Sinha and Julin Maloof at the University of California, Davis
Shu-Ling Chiu	Hollis Cline	Alea A. Mills	Postdoctoral fellow in Dr. Richard Huganir's laboratory at Johns Hopkins University School of Medicine
Keisha John	Linda Van Aelst	Josh Dubnau	Postdoctoral fellow with Dr. Mary Beth Hatten at The Rockefeller University
Jermy Wilusz	David L. Spector	John R. Inglis	Postdoctoral fellow with Dr. Phillip Sharp at the Massachusetts Institute of Technology

2009 THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 2004

Daniel Chitwood, March 4, 2009

Patterning in leaves via a cascade of small RNAs.

Thesis Examining Committee

Chair:	**David Jackson**
Research mentor:	**Marja Timmermans**
Academic mentor:	**Alea A. Mills**
Committee member:	**David L. Spector**
Committee member:	**James Carrington,** *Oregon State University*
External examiner:	**Timothy Nelson,** *Yale University*

Keisha John, March 6, 2009

Characterization of the role of dock7 *in neuronal development.*

Thesis Examining Committee

Chair:	**Alea A. Mills**
Research mentor:	**Linda Van Aelst**
Academic mentor:	**Josh Dubnau**
Committee member:	**Hollis Cline**
Committee member:	**Peter Scheiffele,** *Columbia University*
External examiner:	**Mary Beth Hatten,** *The Rockefeller University*

ENTERING CLASS OF 2005

Jeremy Wilusz, March 23, 2009

3′-end processing of long nucleus-retained noncoding RNAs yields tRNA-like small RNAs.

Thesis Examining Committee

Chair:	**Senthil K. Muthuswamy**
Research mentor:	**David L. Spector**
Academic mentor:	**John R. Inglis**
Committee member:	**Adrian R. Krainer**
Committee member:	**Gordon G. Carmichael,** *University of Connecticut*
External examiner:	**James L. Manley,** *Columbia University*

Teaching Award

At this year's graduation ceremony, Dr. Gregory Hannon was presented with the fourth annual Winship Herr Faculty Teaching Award, named in honor of the School's founding dean. Greg has been an active member of the School's teaching faculty since its inception. He has been the lead instructor of our fall Scientific Reasoning and Logic (SRL) course for the past 4 years and leads the highly innovative Study Section module of the SRL course. He was chosen by the students for this award, based on his enthusiasm, excellence, and creativity in teaching. The following is some of what the students had to say about Greg:

Gregory Hannon

> *The design of the Study Section module itself demonstrates, in part, Greg's teaching creativity. Many graduate programs provide opportunities for students to critically assess science that has already been done, but few require students to look into the future of science and to critically assess the reasoning and logic of proposed experiments in this way.*

> *Greg went beyond what was required to ensure that we all achieved our best in the courses. It is obvious that he teaches because he is passionate and cares about our learning. Even with his very busy schedule he would find the time to ensure that we all got the best learning experience.*

Admissions 2009

The School received 209 applications for the 2009/2010 academic year and is deeply indebted to its Admissions Committee, which reviewed, interviewed, and selected candidates for our doctoral program. The Admissions Committee for the 2009 Entering Class comprised Gregory Hannon (Chair), Josh Dubnau, Leemor Joshua-Tor, Adam Kepecs, Adrian R. Krainer, Zachary Lippman, Robert Lucito, Nicholas Tonks, Lloyd Trotman, and Linda Van Aelst—a truly remarkable team!

DOCTORAL THESIS RESEARCH

Student	Academic mentor	Research mentor	Thesis research
ENTERING CLASS OF 2004			
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	Proteomic analysis to elucidate the splicing and nonsplicing roles of SR proteins.
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	Willliam 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.
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.
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.*
Amy Rappaport *Barbara McClintock Fellow*	William Tansey/ Marja Timmermans	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 e *-myc* model.
ENTERING CLASS OF 2007			
Megan Bodnar *Starr Centennial Scholar*	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*	Bruce Stillman	Gregory Hannon	Recombination hotspots: Characterizing fine-scale variation in the frequency of meiotic recombination across the mammalian genome.

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
Joseph Calarco *David H. Koch Fellow*	David Jackson	Leemor Joshua-Tor/ Robert Martienssen	Mechanistic insights into chromatin regulating proteins in *Schizosaccharomyces pombe* and *Arabidopsis thaliana*.
Saya Ebbesen *Starr Centennial Scholar*	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* *William Randolph Hearst Scholar*	Adrian R. Krainer	Gregory Hannon	A potential role for small RNAs in heterochromatin formation in *Drosophila melanogaster* somatic cells.
Kyle Honegger *Crick-Clay Fellow*	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*	David Jackson	Gregory Hannon	3′UTR role in posttranscriptional regulationof gene expression.
Michael Pautler *William R. Miller Fellow*	Robert Lucito	David Jackson	The RAMOSA pathway and inflorescence branching in maize.
Maria Pineda *Beckman Graduate Student* *William Randolph Hearst Scholar*	Adrian R. Krainer	Raffaella Sordella	Mechanism of "addiction" to receptor tyrosine kinases in non-small-cell lung carcinoma.
Yevgeniy Plavskin *Alfred Hershey Fellow*	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*	Bruce Stillman	Adam Kepecs	Trans-regional coordination of activity in the mouse brain.
Zhenxun Wang *A*STAR Fellow*	Terri Grodzicker	Adrian R. Krainer	Alternative splicing of pyruvate kinase M in tumorigenesis.
Petr Znamenskiy *David and Fanny Luke Fellow*	Terri Grodzicker	Anthony Zador	Pathways for attention and action in the auditory system.
ENTERING CLASS OF 2008			
Philippe Batut *Florence Gould Fellow* Proposal defense: February 2010	Alexander Gann	Thomas Gingeras	Contributions of transposable elements to the evolution of gene regulation in the *Drosophila* phylogeny.
Mitchell Bekritsky *Beckman Graduate Student* Proposal defense: January 2010	W. Richard McCombie	Michael Wigler	Evaluating the puzzle of tumor evolution using single-cell sequencing.
Dario Bressan *Lindsay Fellow* Proposal defense: January 2010	Z. Josh Huang	Gregory Hannon	A genomic approach toward the elucidation of connectivity patterns at cellular resolution in complex neural networks.
Carrie Clendaniel *Beckman Graduate Student* Proposal defense: March 2010	David Jackson	Marja Timmermans	Role of hira in the regulation of develop mentally important target loci.
Melanie Eckersley-Maslin *Genentech Foundation Fellow* Proposal defense: February 2010	Gregory Hannon	David L. Spector	Making the choice: Mechanistic insights into random autosomal monoallelic expression in mammalian cells.
Jiahao Huang *Beckman Graduate Student* Proposal defense: February 2010	Terri Grodzicker	Raffaella Sordella	Network control of epithelial–mesenchymal transition (EMT) progression, maintenance, and reversion.
Sang Geol Koh *George A. and Marjorie H. Anderson Fellow* Proposal defense: February 2010	Glenn Turner	Anthony Zador	Attention-dependent information routing in the mouse auditory cortex.

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
Katie Liberatore *Starr Centennial Scholar* Proposal defense: February 2010	Adrian R. Krainer	Zachary Lippman	The "rules" of floral branching systems: A comparative morphological and molecular study of inflorescence development.
Ozlem Mert *George A. and Marjorie H. Anderson Fellow* Proposal defense: January 2010	John R. Inglis	Scott Lowe	Characterization of the role of E2F7 in oncogene-induced senescence and tumorigenesis.
Elizabeth Nakasone *Leslie C. Quick, Jr. Fellow* *William Randolph Hearst Fellow* Proposal defense: March 2010	Alea A. Mills	Mikala Egeblad	Understanding the effects of cytotoxic chemotherapeutics on the innate immune response.
Zinaida Perova *Charles A. Dana Fellow* Proposal defense: January 2010	Linda Van Aelst	Bo Li	Role of medial prefrontal cortex in behavioral depression.
Felix Schlesinger *Crick-Clay Fellow* Proposal defense: January 2010	Gregory Hannon	Thomas Gingeras	Classification of transcription start sites outside known core promoters.
Nilgun Tasdemir *Lindsay Fellow* Proposal defense: January 2010	Josh Dubnau	Scott Lowe	Investigating the molecular overlaps between epigenetic reprogramming and transformation.
Elvin Wagenblast *Starr Centennial Scholar* Proposal defense: January 2010	Jan A. Witkowski	Gregory Hannon	Role of stem/progenitor cells in mammary gland and breast tumors.
Susann Weissmueller *Annette Kade Fellow* Proposal defense: January 2010	Raffaella Sordella	Scott Lowe	In vivo identification and characterization of tumor suppressor genes in hepatocellular carcinoma.

The Watson School 2009 class enjoying dinner at the home of Dr. and Mrs. Watson. (*Left to right*) Kristen Delevich, Silvia Fenoglio, Ian Peikon, Stephane Castel, James Watson, Kaja Wasik, Cinthya Zepeda Mendoza, Wee Siong Goh

ENTERING CLASS OF 2009

Stephane Castel, University of Guelph
Academic mentor: Lloyd Trotman

Kristen Delevich, University of Pittsburgh
Academic mentor: Bo Li

Silvia Fenoglio, University of Turin
Academic mentor: Linda Van Aelst

Wee Siong Goh, University of Pennsylvania
Academic mentor: Hiro Furukawa

Ian Peikon, Duke University
Academic mentor: Mickey Atwal

Kaja Wasik, Warsaw University
Academic mentor: Jan A. Witkowski

Cinthya Zepeda Mendoza, National Autonomous University of Mexico
Academic mentor: Thomas Gingeras

Entering Class of 2009

On August 24, 2009, the Watson School opened its doors for the 11th time to welcome yet another new class. This year, seven students joined the School: Stephane Castel, Kristen Delevich, Silvia Fenoglio, Wee Siong Goh, Ian Peikon, Kaja Wasik, and Cinthya Zepeda Mendoza. Reflecting CSHL's eclectic mix of nationalities, the entering class of 2009 is also international, hailing from the United States, Canada, Italy, Mexico, Poland, and Singapore.

The 2009 entering class: (*Back, left to right*) Kristen Delevich, Ian Peikon, Stephane Castel, Wee Siong Goh; (*front, left to right*) Silvia Fenoglio, Kaja Wasik, Cinthya Zepeda Mendoza

Academic Mentoring

The Watson School takes great pride in the level of mentoring that it offers its students. One of the very special aspects in this regard is our two-tiered mentoring approach whereby each student receives an academic mentor as well as a research mentor. Entering students select by mutual agreement a member of the research or nonresearch faculty to serve as an academic mentor—a watchful guardian to look over and encourage the student through the sometimes-trying process of their 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 to our success. This year's new academic mentors for the entering class of 2009 are

STUDENT	MENTOR	STUDENT	MENTOR
Stephane Castel	Lloyd Trotman	Cinthya Zepeda Mendoza	Thomas R. Gingeras
Kristen Delevich	Bo Li	Ian Peikon	Gurinder (Mickey) Atwal
Silvia Fenoglio	Linda Van Aelst	Kaja Wasik	Jan A. Witkowski
Wee Siong Goh	Hiro Furukawa		

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 R. Krainer (Chair), David Jackson, Leemor Joshua-Tor, Nicholas Tonks, and Glenn Turner—continues to carefully monitor and develop the curriculum. Glenn replaced Z. Josh Huang, who had served since 2005, on the committee this year. We thank Josh for his input and advice while serving on the committee. 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 Pologruto, 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 on the following page 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 between CSHL and SBU more than 30 years ago. In addition, we often have visiting students from institutions around the world who come to study in our laboratories. 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 students listed in the box below joined us this year.

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS

Student	CSHL research mentor	Affiliation and program
Timour Baslan	James Hicks	Stony Brook, Molecular and Cellular Biology
Matthew Camiolo	Raffaella Sordella	Stony Brook, Genetics
Sudipto Chakrabortty	Thomas Gingeras	Stony Brook, Genetics
Dong-Woo Hwang	Alea A. Mills	Stony Brook, Genetics
Willey Liao	Michael Q. Zhang	Stony Brook, Applied Math and Statistics
Baoyi Liu	Scott Powers	Stony Brook, Genetics
Yifan Mo	Michael Q. Zhang	Stony Brook, Applied Math and Statistics
Vadim Pinskiy	Partha Mitra	Stony Brook, Biomedical Engineering
Jie Wu	Michael Q. Zhang	Stony Brook, Applied Math and Statistics
Xin Zhou	Scott Powers	Stony Brook, Molecular and Cellular Biology

A Major Change to Graduate Student Seminar

Continuing the tradition begun in the summer of 2008, three Graduate Student Symposia were held in 2009 at the Laboratory's Genome Research Center in Woodbury: one each in January, April, and October. Each Symposium consisted of a full day of activities including 20-minute and 10-minute talks by senior students and poster presentations by more junior students. Lunch and a wine and cheese reception rounded out the program and provided opportunities for more informal interactions. As with the inaugural event held in August 2008, the students and faculty in attendance found the Symposia to be very worthwhile events. The Graduate Student 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—Xiaoyun Wu (SBU) and Colin Malone (WSBS)—for their dedication and hard work in helping to refine the format and extend the success of the Graduate Student Symposium and to Judy Wieber for overseeing the administrative elements.

2009 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date	WSBS representatives/titles
Rice University: The Institute of Biosciences and Bioengineering Information Fair	Rice University	January 14	Dawn Pologruto, Director of Admissions and Student Affairs
Wellesley College: Women in Science Fair	Wellesley College	February 25	information sent for distribution
State University of New York, Farmingdale: Information Session and Visit	Cold Spring Harbor Laboratory	May 1	Katie Liberatore, Graduate Student; Hassana Oyibo, Graduate Student; Dawn Pologruto Fred Rollins, Graduate Student
New York City College of Technology: NSF STEP Program Visit	Cold Spring Harbor Laboratory	July 16	Eugene Plavskin, Graduate Student; Dawn Pologruto
23rd Symposium of The Protein Society: Graduate School Fair	Boston, Massachusetts	July 26	Michael Pautler, Graduate Student
University of Medicine and Dentistry, New Jersey: Summer Internship Program Visit	Cold Spring Harbor Laboratory	July 30	Michael Pautler; Dawn Pologruto
University of Puerto Rico, Cayey: Minority Access for Research Careers (MARC) Program Information Session	University of Puerto Rico, Cayey	September 16	Paloma Guzzardo, Graduate Student
Massachusetts Institute of Technology: Career Fair	Massachusetts Institute of Technology	September 17	Eugene Plavskin; Petr Znamenskiy, Graduate Student
University of Puerto Rico, Rio Piedras: Minority Access for Research Careers (MARC) Program Information Session	University of Puerto Rico, Rio Piedras	September 17	Paloma Guzzardo
Massachusetts Institute of Technology: Information Session	Massachusetts Institute of Technology	September 18	Eugene Plavskin; Petr Znamenskiy
Universidad Interamericano de Puerto Rico: Information Session	Universidad Interamericano de Puerto Rico	September 18	Paloma Guzzaardo
Cornell University: Information Session	Cornell University	September 22	Amy Rappaport, Graduate Student; Fred Rollins, Graduate Student
Cornell University: Graduate and Professional School Day	Cornell University	September 23	Amy Rappaport; Fred Rollins
University of Maryland, Baltimore County: Meyerhoff Scholarship Program Visit	University of Maryland, Baltimore County	September 28	Keisha John, Watson School of Biological Sciences Alumna
The Johns Hopkins University: Information Session	The Johns Hopkins University	September 29	Saya Ebessen, Graduate Student
Notre Dame University: Information Session	Notre Dame University	October 1	Carrie Clendaniel, Graduate Student
Northwestern University: Information Session	Northwestern University	October 2	Carrie Clendaniel
Washington University, St. Louis: Information Session	Washington University, St. Louis	October 5	Colin Malone, Graduate Student
Georgetown University: Information Session	Georgetown University	October 6	Dawn Pologruto
Hunter College: Minority Access for Research Careers (MARC) Program Information Session	Hunter College	October 7	Amy Rappaport
University of California, Berkeley: Graduate School Fair	University of California, Berkeley	October 7–8	Colin Malone
University of New Mexico: Information Session	University of New Mexico	October 8	Katie Liberatore
University of Guelph, Canada: Information Session	University of Guelph, Canada	October 8	Joseph Calarco, Graduate Student; Michael Pautler
Barry University: Minority Access for Research Careers (MARC) Program Information Session	Barry University	October 9	Maria Pineda, Graduate Student
University of Toronto: Information Session	University of Toronto	October 9	Joseph Calarco; Michael Pautler
California Institute of Technology: Career Fair	California Institute of Technology	October 14	Colin Malone
Princeton University: Information Session	Princeton University	October 15	Katie McJunkin, Graduate Student
Society for Advancement of Chicanos and Native Americans in Science (SACNAS)	Dallas, Texas	October 15–17	Melanie Eckersley-Maslin, Graduate Student; Katie Liberatore
Princeton University: Graduate and Professional School Fair	Princeton University	October 16	Katie McJunkin
Yale University: Information Session	Yale University	October 22	Kyle Honegger, Graduate Student
Harvard University: Information Session	Harvard University	October 23	Kyle Honegger
University of California, Los Angeles: Graduate School Fair	University of California, Los Angeles	October 28	Elizabeth Nakasone, Graduate Student

2009 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE (*continued*)

Event	Location	Date	WSBS representatives/titles
University of Southern California: Information Session	University of Southern California	October 29	Elizabeth Nakasone
Annual Biomedical Conference for Minority Students (ABRCMS) Graduate Student	Phoenix, Arizona	November 4–7	Paloma Guzzardo; Keisha John; Dawn Pologruto; Hassana Oyibo,
Queensborough Community College: Experiential Learning Conference	Queensborough Community College	November 4	information sent for distribution
Stanford University: Graduate School Fair	Stanford University	November 5	Megan Bodnar, Graduate Student
California Forum for Diversity for Graduate Education: Graduate School Fair	University of California, Santa Cruz	November 7	Megan Bodnar
University of Pennsylvania: Bioengineering/Biotechnology Virtual Career Fair	University of Pennsylvania	November 12–December 15	Virtual Fair
Sigma Xi Annual Conference and Research Symposium	Houston, Texas	November 12–15	Dawn Pologruto
SUNY, Farmingdale: Information Session and Visit	Cold Spring Harbor Laboratory	November 20	Hassana Oyibo; Dawn Pologruto; Eugene Plavskin
Vassar College: Genetics and Bioinformatics Course Visit and Information Session	Cold Spring Harbor Laboratory	December 3	Yaniv Erlich, Graduate Student; Dawn Pologruto; Fred Rollins; Doreen Ware, Assistant Professor
California State University, Sacramento: Science Educational Equity Program	California State University, Sacramento	December 5	information sent for distribution

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 2009:

Postdoctoral Fellows

Victoria Aranda Calleja
Yoselin Benitez Alfonso
Anka Bric
Santanu Chakraborty
Samit Chatterjee
Min Hsuan Chen
Shuaili Chen
Wen Yu Chung
Anne Daulny
Ebru Demir
Katalin Fejes-Toth
Gidon Felsen
Fuqiang Geng
Vladimir Grubor
Nobuhiko Hiramatsu
Katarzyna Hrecka
Bing Hu
Robyn Johnston
William Keyes
Sandra Kuhlman
Claus-Dieter Kuhn
John Kurland
Nancy Liu Sullivan
Jernej Murn
Gonzalo Hugo Otazu Aldana
Ananda Sarkar
Richard Slotkin
Vihra Sotirova
Clinton Whipple
Zuo Zhang

Graduate Students

Allison Blum
Abhishek Chakraborty
Hsueh-Cheng Chiang
Daniel Chitwood
Xingyue He
Keisha John
Danny Khalil
Anna Kloc
Jane Lee-Osborne
Xiaofei Liu
Alexandra Lucs
Hazeem Okunola
Avi Rosenberg
Jeffrey Rosenfeld
Hongjae Sunwoo
Jeremy Wilusz
Wen Xue

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. I wish to thank faculty members Terri Grodzicker, W. Richard McCombie, Alea A. Mills, David L. Spector, Nicholas Tonks, and Linda Van Aelst, who continued to serve on the Executive Committee through 2009. Dick McCombie and Nick Tonks completed their second terms in December 2009. We are grateful for the insights and advice that Dick and Nick brought to the Committee as members for 6 years. As happens each year, there was also turnover among the student representatives. SBU representative Xiaoyun Wu completed her term in September and was

replaced by Matthew Lazarus. WSBS representative Eugene Plavskin also completed his term in December. We are thankful to Xiaoyun and Eugene for their honest and thoughtful advice.

Special Events: The Gavin Borden Fellow

The annual Gavin Borden Visiting Fellow (so named after the energetic and charismatic publisher of *Molecular Biology of the Cell*, who died of cancer in 1991) brings to CSHL an eminent researcher and educator to give the Gavin Borden Lecture, which is dedicated to the graduate students at the laboratory. Dr. Ralph J. Greenspan—the Dorothy and Lewis B. Cullman Fellow in Experimental Neurobiology at The Neurosciences Institute in San Diego—was this year's Gavin Borden Fellow. His lecture *Genes and Behavior: Is There a Unifying Principle, or Is It Just a Lot of Stuff?* was thoroughly enjoyed and evoked many questions from the audience. In addition, Ralph shared his experiences as a scientist during dinner as well as at a roundtable lunch and discussion with the graduate students the following day.

The Watson School Continues to Benefit from Generous Benefactors

We are very pleased to announce the granting of a new fellowship from the Genentech Foundation to the WSBS. This fellowship will be used to support the educational and research costs for one student for a period of 3 years. We also hope that by creating this relationship with Genentech, the entire WSBS community will benefit from the connection to a leader in the biotechnology industry.

In 2008, we established the Gonzalo Río Arronte Fellowship, to be held by a qualified Mexican graduate student at the WSBS. This year, I am pleased to report the appointment of Ms. Cinthya Zepeda Mendoza as the first recipient. Cinthya received her Bachelor's degree from the Undergraduate Program in genomic sciences at the National Autonomous University of Mexico and joined the WSBS entering class of 2009 in August. As part of her fellowship, Cinthya will be expected to have a role in the development of genomic sciences in Mexico on completion of her graduate and postdoctoral training.

We are extremely grateful to our generous donors, whose one-time gifts or continued support made our 2009 programs possible, including Abrams Charitable Trust, an anonymous donor, The Banbury Fund, The Arnold and Mabel Beckman Foundation, Bristol-Myers Squibb Corporation, Mr. and Mrs. Richard M. Cashin, Mr. and Mrs. Landon Clay, Lester Crown, The Dana Foundation, The William Stamps Farish Fund, Mr. and Mrs. Alan Goldberg, Florence Gould Foundation, William Randolph Hearst Foundation, Dr. Mark Hoffman, Annette Kade Charitable Trust, Mr. David H. Koch, Mr. and Mrs. Robert D. Lindsay and Family, Mr. and Mrs. David Luke III, Medicus Polish American Society, Mr. and Mrs. William R. Miller, The Millipore Foundation, The Quick Family, The Rathmann Family Foundation, and The Roy J. Zuckerberg Family Foundation. We are also very fortunate to hold a National Research Service Award Predoctoral Training Grant from the National Institutes of Health, National Institute of General Medical Sciences.

Student Achievements

The WSBS students continue to impress us all with 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 140 papers to date, a remarkable accomplishment for a school in its 11th year. In addition, our current students and alumni have been successful in receiving prestigious awards and fellowships. In 2009, Jeremy Wilusz was awarded the RNA Society/Scaringe Award. Three current students—Joseph Calarco, Michael Pautler, and Stephane Castel—were all awarded Graduate Scholarships from the Natural Sciences and Engineering Research Council of Canada (NSERC). WSBS alum Patrick Paddison was named a Pew Scholar in the Biomedical Sciences. WSBS graduate Elizabeth Murchsion received a L'Oreal UNESCO UK and Ireland Fellowship For Women In Science, and WSBS graduate Christopher Harvey received a Helen Hay Whitney Postdoctoral Fellowship.

2009 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS

Asari H, Zador AM. 2009. Long-lasting context dependence constrains neural encoding models in rodent auditory cortex. *J Neurophysiol* **102:** 2638–2656.

Benitez-Alfonso Y, **Cilia M**, Roman AS, Thomas C, Maule A, Hearn S, Jackson D. 2009. Control of *Arabidopsis* meristem development by thioredoxin-dependent regulation of intercellular transport. *Proc Natl Acad Sci* **106:** 3615–3620.

Blum AL, Li W, Cressy M, Dubnau J. 2009. Short- and long-term memory in *Drosophila* require cAMP signaling in distinct meuron types. *Curr Biol* **19:** 1341–1350.

Bolduc FV, Tully T. 2009. Fruit flies and intellectual disability. *Fly* **3:** 91–104.

Bric A, Miething C, Bialucha CU, **Scuoppo C**, Zender L, Krasnitz A, Xuan Z, Zuber J, Wigler M, Hicks J, McCombie RW, Hemann MT, Hannon GJ, Powers S, Lowe SW. 2009. Functional identification of tumor-suppressor genes through an in vivo RNA interference screen in a mouse lymphoma model. *Cancer Cell* **16:** 324–335.

Chitwood DH, Nogueira FTS, Howell MD, Montgomery TA, Carrington JC, Timmermans MCP. 2009. Pattern formation via small RNA mobility. *Genes Dev* **23:** 549–554.

Collins G, Lipford R, Deshaies RJ, Tansey WP. 2009. Gal4 turnover and transcription activation. *Nature* **461:** E7–E7.

Czech B, Zhou R, **Erlich Y**, Brennecke J, Binari R, Villalta C, Gordon A, Perrimon N, Hannon GJ. 2009. Hierarchical rules for Argonaute loading in *Drosophila*. *Mol Cell* **36:** 445–456.

Couvillion MT, Lee SR, Hogstad B, **Malone CD**, Tonkin LA, Sachidanandam R, Hannon GJ, Collins K. 2009. Sequence, biogenesis, and function of diverse small RNA classes bound to the Piwi family proteins of *Tetrahymena thermophila*. *Genes Dev* **23:** 2016–2032.

Erlich Y, Chang K, Gordon A, Ronen R, Navon O, Rooks M, Hannon GJ. 2009. DNA Sudoku—Harnessing high-throughput sequencing for multiplexed specimen analysis. *Genome Res* **9:** 1243–1253.

Husbands AY, **Chitwood DH**, **Plavskin Y,** Timmermans MCP. 2009. Signals and prepatterns: New insights into organ polarity in plants. *Genes Dev* **23:** 1986–1997.

Lima SQ, Hromádka T, **Znamenskiy P**, Zador AM. 2009. PINP: A new method of tagging neuronal populations for identification during in vivo electrophysiological recording. *PLoS One* **4:** e6099.

Makino H, Malinow R. 2009. AMPA receptor incorporation into synapses during LTP: The role of lateral movement and exocytosis. *Neuron* **64:** 381–390.

Malone CD, Hannon GJ. 2009. Small RNAs as guardians of the genome. *Cell* **136:** 656–668.

Malone CD, Brennecke J, **Dus M**, Stark A, McCombie WR, Sachidanandam R, Hannon GJ. 2009. Specialized piRNA pathways act in germline and somatic tissues of the *Drosophila* ovary. *Cell* **137:** 522–535.

Nogueira FT, **Chitwood DH**, Madi S, Ohtsu K, Schnable PS, Scanlon MJ, Timmermans MC. 2009. Regulation of small RNA accumulation in the maize shoot apex. *PLoS Genet* **1:** e1000320.

Rechavi O, **Erlich Y**, Amram H, Flomenblit L, Karginov FV, Goldstein I, Hannon GJ, Kloog Y. 2009. Cell contact-dependent acquisition of cellular and viral nonautonomously encoded small RNAs. *Genes Dev* **23:** 1971–1979.

Sunwoo H, Dinger ME, **Wilusz JE,** Amaral PP, Mattick JS, Spector DL. 2009. MEN ∈/β nuclear retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles. *Genome Res*. **19:** 347–359.

Wilusz JE, Sunwoo H, Spector DL. 2009. Long noncoding RNAs: Functional surprises from the RNA world. *Genes Dev* **23:** 1494–1504.

Zuber J, Radtke I, Pardee TS, Zhao Z, **Rappaport AR**, Luo W, McCurrach ME, Yang M-M, Dolan ME, Kogan SC, Downing JR, Lowe SW. 2009. Mouse models of human AML accurately predict chemotherapy response. *Genes Dev* **23:** 877–889.

WSBS Family Events

Finally, I am pleased to announce that this was a great year for both the WSBS students and administration as we celebrated some wonderful personal occasions. On May 13, student Eyal Gruntman, his wife Neomi, and son Jonathan welcomed baby Eli. On May 16, we celebrated the marriage of Dawn Meehan to Thomas Pologruto. In addition, Kyle Honegger (entering class of 2007) became engaged. Our congratulations and best wishes to all of them.

Leemor Joshua-Tor
Professor and Dean

Eli Gruntman, Eyal and Neomi's new baby boy!

Marriage of Dawn Meehan and Thomas Pologruto

SPRING CURRICULUM

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 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 2009, there were two such courses: Evolution and Microbial Pathogenesis.

Evolution

Attended by the entering classes of 2007 and 2008

INSTRUCTOR **Nipam Patel,** University of California, Berkeley

VISITING LECTURERS **Rob DeSalle,** American Museum of Natural History
David Linberg, University of California, Berkeley
Mike Palopoli, Bowdoin College

TEACHING FELLOWS **Crystal Chaw,** University of California, Berkeley
Brian Kraatz, University of California, Berkeley
Anna-Sapfo Malaspinas, University of California, Berkeley
Brian Moore, University of California, Berkeley

The field of evolutionary biology touches upon all other areas of the biological sciences, because every form of life and every biological process represent an ongoing evolutionary "experiment." Our aim in this course was to both discuss our understanding of the mechanisms of evolution and explore how evolutionary data can be used to further our understanding of various biological problems.

The course began with a discussion of the diversity of organisms that currently exist and our methods for understanding the evolutionary relationships between these organisms. We then went on to study how paleontological data are collected and used to understand the history of life on earth. We then examined how DNA sequence data can be used to understand the evolutionary history of organisms, genes, and genomes. Within this molecular and genetic framework, our focus shifted to the mechanisms of evolutionary change and how variation within populations leads to the evolution of new species. Finally, we discussed how morphological changes are brought about through evolutionary changes in development and gene regulation. The hope was that this course would provide the student with a general overview of evolutionary biology and how to use evolutionary data to gain further insight into all manner of biological problems. The course ran from Saturday, March 28, to Sunday, April 4 and included a class favorite field trip and curator tour at

Evolution course participants: (*Front row, left to right*) Philippe Batut, Dario Bressan, Crystal Chaw, Carrie Clendaniel, Maria Pineda, Mitchell Bekritsky, Beth Nakasone, Zina Perova, Jiahao Huang, Joe Calarco. (*Middle row, left to right*) Marek Kudla, Saya Ebbesen, Brian Moore, Anna-Sapfo Malaspinas, Katie Liberatore, Ozlem Mert, Melanie Eckersley Maslin. (*Back row, left to right*) Ralph Burgess, Kyle Honegger, Josh Sanders, Felix Schlesinger, Megan Bodnar, Mike Pautler, Nilgun Tasdemir, Paloma Guzzardo, Hassana Oyibo, Zhenxun Wang, Petr Znamentskiy, Sang-Geol Koh, Eugene Plavskin, Elvin Wagenblast, Susann Weismeuller, Hernan Garcia, Michael Palopoli.

the American Museum of Museum of Natural History in New York City. As in previous years, the course was highly rated by all of the students.

Microbial Pathogenesis

Attended by the entering classes of 2005 and 2006

INSTRUCTORS	**Stanley Maloy,** Center for Microbial Sciences, San Diego **Ronald K. Taylor,** Dartmouth Medical School
VISITING LECTURERS	**Heran Darwin,** New York University **Darren Higgins,** Harvard Medical School **Linda Kenney,** University of Illinois, Chicago

Throughout recorded history, microbial pathogens have been a major cause of human disease and mortality. However, with the advent of effective antibiotics, it seemed like the war on microbes had been won. Hence, for several decades, health-related research shifted to topics like cancer, heart disease, and genetic diseases. Although research in microbial pathogenesis slowed, the microbes demonstrated the efficacy of evolution. Microbial resistance to antibiotics developed faster than new antibiotics could be made available, and the resistance spread throughout the microbial world. The global expansion of food distribution networks has increased the incidence of common microbial pathogens. Simultaneously, emerging microbial pathogens filled new ecological niches, such as indwelling medical devices that provide a surface for biofilms and the growing population of patients who are immunocompromised due to primary infections such as HIV or due to therapies for chronic diseases. Furthermore, recent discoveries have demonstrated that some diseases (e.g., ulcers) previously believed

to be caused by a genetic predisposition or environmental conditions are actually caused by microbes. This microbial offensive has summoned a renewed counterattack on microbial pathogens that has intensified during the last several years. Meanwhile, a variety of new tools have become available that make it possible to dissect the molecular basis of pathogenesis both from the microbial and host perspectives. Recently, the complete DNA sequence of bacterial pathogens has provided valuable insights into how microbial pathogens evolve and the extent of gene transfer between pathogens. These advances have revealed new ways to control infection, including the identification of novel targets for antimicrobials and novel approaches for vaccine development. Nevertheless, many more questions remain unanswered and many pathogens are still poorly understood.

Understanding bacterial pathogenesis demands a detailed knowledge of the host response as well as the pathogen itself. Both of these perspectives provide potential strategies for solving important clinical problems. To elucidate these distinct aspects of microbial pathogenesis requires an interdisciplinary approach, integrating the fields of microbiology, eukaryotic cell biology, immunology, and genomics.

This course focused on mechanisms of microbial pathogenesis and the host response, and the scientific approaches that are used to investigate these mechanisms. How do microbes adhere to host cells? How do environmental cues direct the response of microbial pathogens? How do microbial pathogens modulate host cells to expedite virulence? How do host cells respond to microbial pathogens? How does the host immune system react to microbial pathogens? What does genomics tell us about how microbial pathogens evolve? How do emerging pathogens take advantage of new ecological niches? Although there are numerous microbial pathogens, the answers to these questions indicate that many pathogens use similar approaches to solve common problems. The course integrated lectures by the instructors, directed readings of research papers, and seminars by the instructors plus three invited speakers who specialize in various aspects of bacterial pathogenesis. The course ran from Saturday, April 19, through Sunday, April 25. The students rated this course as one of their favorites.

Microbial Pathogenesis course participants: (*Front row, left to right*) Karen Liu, Claudio Scuoppo, Yaniv Erlich, Amy Leung, Katie McJunkin. (*Middle row, left to right*) Heran Darwin, Amy Rappaport, Oliver Tam, Hiroshi Makino. (*Back row, left to right*) Stanley Maloy, Fred Rollins, Jeremy Wilusz, Colin Malone, Patrick Finigan, Ronald Taylor.

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR	**David A. Micklos**	
INSTRUCTORS	**Amanda McBrien (Lead)**	**Jermel Watkins**
	Jennifer Cutillo	**Elna Gottlieb**
	Jason Williams	**Erin McKechnie**
ADMINISTRATOR	**Carolyn Reid**	

As science plays an increasing role in society, there is also an increasing need for biologists to educate nonscientists of all ages about biology. The Watson School of Biological Sciences doctoral program offers its students unique teaching experiences through the Laboratory's Dolan DNA Learning Center, where students teach laboratory courses to high school and middle school students. From these teaching experiences, they learn how to communicate with nonbiologists and to inspire and educate creative young minds. The teaching module entailed pairs of students teaching one morning or afternoon a week for 12 weeks. In the initial weeks, the Dolan DNA Learning Center instructors taught the Watson School students the didactic process; it was not until the 5th week that the graduate students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty the opportunity to get to know each other and to explore possibilities for doctoral thesis research. At the end of each rotation, students make short presentations of their studies to the other students and their rotation advisors. These talks give students an opportunity to share their laboratory experiences and to learn how to give a scientific presentation. With this latter goal in mind, in addition to the research mentors, the instructors of the Scientific Exposition and Ethics core course and members of the School's Executive Committee attend the talks and give individual feedback to students on their presentations. This year, 22 WSBS faculty members and one research professor served as rotation mentors to our largest class to date, some mentoring more than one student.

ROTATION MENTORS	**Mickey Atwal**	**Adrian R. Krainer**	**Bruce Stillman**
	Josh Dubnau	**Bo Li**	**Marja Timmermans**
	Mikala Egeblad	**Zach Lippman**	**Glenn Turner**
	Thomas Gingeras	**Scott Lowe**	**Linda Van Aelst**
	Gregory Hannon	**Robert Martienssen**	**Doreen Ware**
	James Hicks	**Alea A. Mills**	**Michael Wigler**
	Josh Huang	**Raffaella Sordella**	**Anthony Zador**
	Adam Kepecs	**David L. Spector**	

FALL 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
Mikala Egeblad
Grigori Enikolopov
Hiro Furukawa
Adam Kepecs
Bo Li
Robert Martienssen
Thomas Schalch (postdoctoral fellow)
Raffaella Sordella
Bruce Stillman
Lloyd Trotman

VISITING LECTURERS
Elizabeth Lacy, Memorial Sloan-Kettering Cancer Center
William Merrick, Case Western Reserve University

A fundamental aspect of earning the Ph.D. is training in the pursuit of knowledge. In this core course, which forms the heart of the 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 of which had a different theme. Each week, students read an assigned set of research articles and at the end of the module, 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	Macromolecular Structure and Function	Module 4	Signaling in Development
Module 2	Gene Expression	Module 5	Neuroscience
Module 3	Cell Proliferation and Cancer	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
David J. Stewart (Lead)
Alea A. Mills
Arne Stenlund

TEACHING ASSISTANT **Emily Hodges**

GUEST LECTURERS **Walter Goldschmidts**
Jan A. Witkowski

VISITING LECTURERS **Misha Angrist,** Duke University
Olga Akselrod, The Innocence Project
Robert Charrow, Greenberg Traurig, LLP
Avner Hershlag. North Shore University Hospital
Boyana Konforti, Editor, Nature Structural & Molecular Biology
Michael Zigmond, University of Pittsburgh

The 2009 Scientific Exposition and Ethics (SEE) core course content remained essentially the same as in 2008. David Stewart, an instructor last year, took over as lead instructor. Alea Mills, last year's lead instructor, and Arne Stenlund, also returning from last year, were the other instructors. The faculty instructors were again joined by a postdoctoral fellow, Emily Hodges, as a teaching assistant, a format our students have taken to 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 on society as well as the nature and boundaries of ethical behavior of scientists and their rights and responsibilities. A primary objective of the course was that students consider exposition and ethics an integral part of scientific research.

Research Topics

ORGANIZERS **Kimberley Geer**
Alyson Kass-Eisler

This core course provided students with an in-depth introduction to the fields of research that the Laboratory scientists investigate. Students and faculty attended a weekly Research Topics seminar, at which faculty members and CSHL fellows presented their current research topics and methods of investigation each Wednesday evening over dinner. The students learned how to approach important problems in biology. These seminars, together with the annual fall Laboratory In-House Symposium, provided students with a basis for selecting laboratories in which to do rotations. The weekly speakers were

Florin Albeanu
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
Alexander Krasnitz
Bo Li
Zachary Lippman
Scott Lowe
Robert Martienssen
W. Richard McCombie
Alea A. Mills
Partha Mitra
Pavel Osten
Darryl Pappin
Scott Powers
Stephen Shea
Raffaella Sordella
David L. Spector
Arne Stenlund
Bruce Stillman
Marja Timmermans
Lloyd Trotman
Glenn Turner
Christopher Vakoc
Linda Van Aelst
Doreen Ware
Michael Wigler
Anthony Zador
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 from last year, with just some changes to the guest and visiting lecturers.

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 LECTURERS	**Mickey Atwal** **Zachary Lippman**
VISITING LECTURER	**Abraham Palmer,** University of Chicago

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 catalogued, 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	**Florin Albeanu** **Alexei Koulakov**
VISITING LECTURER	**Mayank Mehta, Brown University**

This course provided an overview of key aspects neuroscience. The emphasis was on spanning levels: How can we go from molecules through cells and circuits to behavior? There were three main com-

ponents 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 spanned levels, for example, from the molecular to the synaptic or 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	**Mikala Egeblad** **Darryl Pappin** **David L. Spector** **Bruce Stillman**
VISITING LECTURERS	**Gregg Gundersen,** Columbia University **Alexey Khodjakov,** Wadsworth Laboratory **Timothy McGraw,** Weill Cornell Medical College **Elizabeth Miller,** Columbia University **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 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 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. The Laboratory currently employs ~140 postdoctoral fellows working in the labs of 43 principal investigators. Current fields of research expertise include genetics; molecular, cellular and structural biology; neuroscience; cancer; plant biology; 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 the Laboratory administration to coordinate and organize educational and career development activities. Dr. Alyson Kass-Eisler, the Postdoctoral Program Officer, and Dr. Nicholas Tonks, the Scientific Director of the Postdoctoral Program, provide the day-to-day support for the postdoctoral program, which is overseen by the Dean of the Watson School, Dr. Leemor Joshua-Tor.

In 2009, we held a number of courses and workshops aimed at postdoctoral fellows. On January 28, we were fortunate to host Dr. Colin Goddard, Chief Executive Officer at OSI Pharmaceuticals, who talked to the students about "Marrying Bio Science & Business: The Changing Face of Biotech." In this lively discussion, Dr. Goddard talked about the difficulties and joys of a career in biotechnology and fielded questions about the industry from the student participants.

On April 13, the Watson School presented a screening of the documentary film "*Naturally Obsessed: The Making of a Scientist*." The film is a true story about the struggle to become a scientist and the satisfaction of discovery. This 1-hour film was produced and directed by Carole Rifkind and her husband, Dr. Richard Rifkind, who were present during the screening and gave a "Q&A" session after the film.

The first annual National Postdoc Appreciation Day was held on September 24. To celebrate, CSHL hosted a lecture from Dr. Kathleen Flint, Project Manager at the National Postdoctoral Association, on "Getting the Most Out of Your Postdoc Experience." Following the lecture, we took advantage of the beautiful weather and enjoyed pizza in the new Hillside Campus courtyard.

On October 2, we brought back the very popular course on grant writing. The first part, led by Walter Goldschmidts and his CSHL Office of Sponsored Research (OSP) staff, focused on introductory grant writing and the grant application process. A new session on identifying postdoctoral fellowships was added this year and included presentations by Dr. Jill Hemish from OSP, Dr. Alyson Kass-Eisler, and postdoctoral fellow Dr. Vishal Thapar. CSHL faculty members W. Richard McCombie and Linda Van Aelst gave an overview of the grant review process with their presentation, "True Confessions of Peer Review." The last part of the program was an effective writing session led by Dr. Philip Vassallo, author of *The Art of On-The-Job Writing*, who also did one-on-one writing consultations with fellows.

Leadership Training was held October 13 and 14 for postdocs who are beginning to plan for their next career step. Sessions on "Motivating and Coaching Your Lab Team," "Creating a Professional Lab," and "Dealing with Interpersonal Conflict" were presented by Dr. Lee Ginsburg from Miller Ginsburg Management Consulting. These sessions were extremely useful for those postdocs who are about to start their own laboratories.

Each year, Laboratory President Dr. Bruce Stillman holds a "Town Hall" meeting specifically for postdoctoral fellows. This year's Town Hall meeting took place on December 8. Dr. Stillman took the opportunity to highlight the generous benefits our postdocs receive. The fellows took the opportunity to talk about what they think could be improved at the Lab. One area that would make life easier for the postdoctoral fellows would be to increase shuttle service at the Laboratory. The Meetings and Courses office will be working with the fellows to implement some of the suggestions.

CSHL has been a member of a special ambassador recruitment program from Merck Research Laboratories (MRL) since 2005. This program provides the 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. As a result of this program, Dr. Zuo Zhang, a postdoctoral fellow from Dr. Adrian Krainer's laboratory, joined MRL as a Research Scientist in 2009. The relationship between MRL and CSHL has been extremely beneficial, and we will be looking toward building additional relationships in the future. Drs. Li and Hoek will be visiting again in January 2010.

Four 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. Each "prize" is given in the form of an educational grant of $500. 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. This year, 16 posters were presented for the postdoctoral prize, which was won by Dr. Anirban Paul, a postdoc in Z. Josh Huang's laboratory.

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 one-on-one tutoring 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 teaching assistants. These two fellows were an integral part of the course by providing their expertise in discussions, editing of the students writing work, and critiquing oral presentations. This format was highly successful and was used again in Fall 2009.

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. On July 29, we were fortunate to host Dr. Robert Tillman, Director of the Science Alliance, for a talk on "Need a doctor? Career first-aid for Ph.D.s." Dr. Tillman also did a one-on-one resume clinic for postdoctoral fellows.

Every other year, the NYAS, in partnership with area institutions including CSHL, hosts "What can you be with a Ph.D?", a 2-day science and technology career symposium. The symposium took place at New York University on November 13–14. The event featured talks by leaders in the pharmaceutical and biotechnology industries, science entrepreneurs, media, government, and academia. It also included exhibitor booths and a networking reception.

We are extremely proud that our postdoctoral fellows have also been successful in obtaining prestigious individual fellowships and awards. Alexei Aravin, a postdoctoral fellow in Gregory Hannon's lab, was named a finalist for the New York Academy of Science's Blavatnik Award, and Erkan

Karakas, from Hiro Furukawa's lab, received CSHL's Harvey L. Karp award. Current postdocs also hold fellowships from 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; Lauri Strauss Leukemia Foundation; NARSAD; National Institutes of Health; 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 United States Department of Defense. Three CSHL postdoctoral fellows have also been fortunate to secure career development funding through the very competitive National Institutes of Health's Pathways to Independence 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 in cancer to the National Institutes of Health in May. We hope to receive funding for this exciting program in 2010.

Finally, a most important measure of our postdoctoral program's success is the ability of postdoctoral fellows to secure permanent positions at the end of their training. In 2009, the Laboratory's departing postdoctoral fellows went on to positions at Brigham Young University, Bristol Myers Squibb, California Institute of Technology, Centre for Genomic Regulation (Barcelona, Spain), Duke University, Memorial Sloan-Kettering Cancer Center, Merck Research Laboratories, National Institute of Plant Genome Research (India), Ohio State University, St. Jude Children's Research Hospital, University of Colorado, and Wake Forest School of Medicine to name just a few.

UNDERGRADUATE RESEARCH PROGRAM

PROGRAM DIRECTOR **David Jackson**

PROGRAM ADMINISTRATOR **Dawn Pologruto**

Each summer, 20–25 undergraduates from around the world and across the country participate in the CSHL summer Undergraduate Research Program (URP). More than 740 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, URPs 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 from CSHL faculty members and outside faculty members including, in 2009, talks by Dr. Stanley Maloy, San Diego State University, and Dr. Alfred Goldberg, Harvard University. URPs also attend a Bioin-

The 2009 Design Your Own Tee Shirt

formatics Workshop Series, where they learn how to identify patterns in DNA and protein sequences and how to interpret them. Lectures by CSHL faculty are specifically designed for URPs. URPs were also invited to join Dr. and Mrs. Watson for a pizza party and Dr. and Mrs. Stillman for dinner. BBQ 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 second year the URPs beat the faculty team in volleyball—quite an accomplishment!

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 528 applicants, took part in the 2009 program:

Christopher Bennett, McGill University
Advisor: **Dr. Adrian R. Krainer**
Sponsor: Robert H.P Olney Fund Fellowship
Presence of intronic splicing silencers downstream from 5′ splice sites.

Philippa Borrill, University of Cambridge
Advisor: **Dr. David Jackson**
Sponsor: C. Bliss Memorial Fund Fellowship
Cell-to-cell trafficking of transcription factor KNOTTED1/SHOOTMERISTEMLESS: Why and how?

Marcela Carmona, University of Pennsylvania
Advisor: **Dr. Marja Timmermans**
Sponsor: National Science Foundation and Department of Defense
The contribution of polarity determinants to organ development.

Philip Coffman, University of New Mexico
Advisor: **Dr. Partha P. Mitra**
Sponsor: Steamboat Foundation Fellowship
Completing the circuit: A practical technique to trace long-range projections in the brain.

Danielle Feldman, Hunter College
Advisor: **Dr. Josh Dubnau**
Sponsor: Hunter College Fund Fellowship and National Science Foundation and Department of Defense
Expression and localization of lightoid (Beck-1) in the *Drosophila melanogaster* central nervous system.

Emma Fink, Amherst College
Advisor: **Dr. David L. Spector**
Sponsor: James D. Watson Fund Fellowship
Tracking the subcellular localization of Malat1, a long ncRNA, in live cells.

Daniel Goltz, Whitman College
Advisor: **Dr. W. Richard McCombie**
Sponsor: National Science Foundation and Department of Defense
Targeted resequencing of the synaptome genes using microarray exon capture.

Debbie Goodman, Columbia University
Advisor: **Dr. Gregory Hannon**
Sponsor: William Townsend Porter Foundation Fellowship
microRNAs regulating gene expression in muscular differentiation.

Mark Grabois, Columbia University
Advisor: **Dr. Adam Kepecs**
Sponsor: Former URP Fund Fellowship
Roles of cholinergic basal forebrain projections in attention.

Lisa Lam, University of California, Berkeley
Advisor: **Dr. Robert Martienssen**
Sponsor: Former URP Fund Fellowship
Reestablishment of silencing events in *Schizosaccharomyces pombe*.

Aviva Mail, Cornell University
Advisor: **Dr. Michael Q. Zhang**
Sponsor: National Science Foundation and Department of Defense
Detection of genomic structural variation from high-throughput sequence data.

Michael Mitchell, University of Arizona
Advisor: **Dr. Bruce Stillman**
Sponsor: 30th Anniversary URP Fund Fellowship
Elucidating the role of DDX5 in cell proliferation and its regulation of essential genes.

Alan Rodriguez Penney, University of Puerto Rico, Rio Piedras
Advisor: **Dr. Bo Li**
Sponsor: National Science Foundation and Department of Defense
Effect of ketamine on striatum and nucleus accumbens neuronal activity.

Julia Rogers, Yale University
Advisor: **Dr. Leemor Joshua-Tor**
Sponsor: National Science Foundation and Department of Defense
Transducer and repressor complex in the yeast GAL induction system.

Natalie Straight, Carnegie Mellon University
Advisor: **Dr. Z. Josh Huang**
Sponsor: Dorcas Cummings Fund Fellowship
Characterizing cell adhesion molecules in GABAergic synapses: Neurexin and neuroligin.

Tim Wang, University of California, Berkeley
Advisor: **Dr. Scott Powers**
Sponsor: Former URP Fund Fellowship
An investigation of the liver cancer epigenome.

Katie Washington, Notre Dame University
Advisor: **Dr. Raffaella Sordella**
Sponsor: University of Notre Dame URP Fund Fellowship
Molecular mechanism of EGFR addiction in H4006 non-small-cell lung carcinoma.

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 chairperson may nominate up to three students. The top candidates are interviewed by CSHL scientists and up to 10 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 10 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

2009/2010 Partners for the Future participants: (*Left to right*) Benjamin Hartman, Lipsa Panda, Sabrina Layna,

in a laboratory. 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 2009–2010 Partners for the Future were chosen from among 16 semifinalist nominations and they are listed below:

Partner	High School	CSHL Mentor	Laboratory
Nikita Anand	Cold Spring Harbor High School	Gregory Hannon	Gregory Hannon
Benjamin Hartman	North Shore High School	Mikala Egeblad	Mikala Egeblad
Sabrina Layne	Oyster Bay High School	Kate Creasey	Robert Martienssen
Norah Liang	Patchogue–Medford High School	Alea A. Mills	Alea A. Mills
Steven McGarty	Cold Spring Harbor High School	Partha Mitra	Partha Mitra
Lipsa Panda	Syosset High School	Morgan Xu	David Jackson
Joshua Weiss	Smithtown High School East	Mickey Atwal	Mickey Atwal

MEETINGS AND COURSES

ACADEMIC AFFAIRS

The Meetings and Courses program at the Laboratory serves to communicate new discoveries, concepts, and methodologies to an international community of scientists. The program consists of advanced laboratory and lecture courses, as well as large meetings and biotechnology conferences that are held almost year round. More than 8000 scientists ranging from graduate students and postdoctoral fellows to senior faculty come from around the world to attend these events at Cold Spring Harbor. A growing international program complements the main program of meetings and courses.

In 2009, the 27 laboratory and lecture courses that were held at the Laboratory attracted more than 1180 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 remains 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.

L. Stein and R. Martienssen at the Personal Genomes Meeting

Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor. Their excellence and dedication make the course program work so well. We would especially like to thank Drs. Karen Adelman, Thomas Brutnell, Beverley Clark, Christopher Fall, Mark Farrant, Vivian Irish, Elizabeth Toby Kellogg, Partha Mitra, Jennifer Normanly, Thomas Oelgeschlager, Randall Smith, and Malcolm Whiteway, some of whom retired this year after many years of service.

Grants from a variety of sources support the courses. The 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 by 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, which brought together almost 7000 scientists from around the world to discuss their latest research. The 74th *Cold Spring Harbor Symposium on Quantitative Biology* on "Evolution: The Molecular Landscape" was dedicated to Charles Darwin on the occasion of the bicentennial of his birth and the 150th anniversary of the publication of *On the Origin of Species.* Opening night speakers included Janet Browne, Ed Wilson, David Kingsley, and Marc Hauser, and Douglas Wallace presented the Reginald Harris lecture on "Energetics in Eukarotic and Human Origins." Kevin Padian enlightened a mixed audience of scientists and lay friends and neighbors with his Dorcas Cummings lecture on "Darwin, Dover & Intelligent Design," and Brian Charlesworth ended the meeting with a masterful and thought-provoking summary. Special sessions were held on "Cultural Evolution," including presentations on principles of natural selection applied to linguistics, ideas, and economics by Daniel Dennett, Matt Ridley, and Niall Ferguson, and on "Evolution and the Public," in which Kevin Padian, Ken Miller, Barbara Forrest, and Eugenie Scott discussed so-called "intelligent design" and the threat such irrational and anti-scientific attitudes pose to education in the United States.

C. Ponting and E. Birney at the Genome Informatics meeting

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 The Biology of Genomes, Retroviruses, and Mechanisms of Eukaroytic Transcription, and Neurobiology of *Drosophila* were all oversubscribed, and many others attracted strong or record attendances. New meetings on Molecular and Cellular Biology of Plasminogen Activation and Single-cell Techniques were held this year. Many of these meetings have become essential for those in the field and are held on a biannual basis. Partial support for individual meetings is provided by grants from the NIH, NSF, foundations, and companies. Core support for the meetings program is provided by the Laboratory's Corporate Sponsor Program.

The joint Cold Spring Harbor/Wellcome Trust conference series held at the genome campus south of Cambridge, England, included four meetings, on Mouse Genetics and Genomes: Development and Diseases; Pharmacogenomics; Integrated Approaches to Brain Complexity; and Engineering Principles. These conferences follow the Cold Spring Harbor model in that the majority of talks are selected from the abstracts. They attracted 367 participants in total.

Finally, 2009 saw the continued development of the Cold Spring Harbor Asia program, as a wholly owned affiliate. An inaugural Banbury-style meeting on Large-scale Planting of Transgenic Crops in Asia: Policy Issues, Scientific Advances, Regulatory Considerations, and Economic Benefits was held at an adjacent facility in Suzhou, China, in November and attracted a highly international group of more than 70 scientists, policy specialists, and economists from academia, industry, and government. CSH Asia's Corporate Advisory Board also met to advise the Laboratory and its affiliate 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 CSHL staff and faculty who contribute their expertise, efforts, and good humor to the program. We thank IT coordinator Taimur Arif, who left CSHL for pastures new, for his dedication and service toward the program.

Terri Grodzicker
Dean
Academic Affairs

David Stewart
Executive Director
Meetings and Courses Program

E. Koonan at the 74th CSHL Symposium on Quantitative Biology

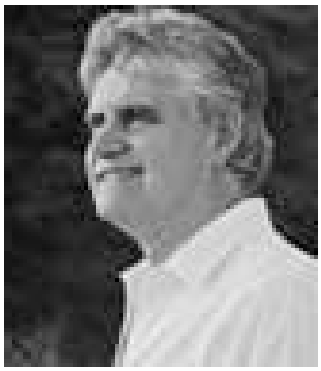
M. Timmermans and M. Bevin at the Plant Genomics meeting

74TH COLD SPRING HARBOR LABORATORY SYMPOSIUM ON QUANTITATIVE BIOLOGY

Evolution: The Molecular Landscape

May 27–June 1 400 Participants

ARRANGED BY **David Stewart, Bruce Stillman, and Jan Witkowski**
Cold Spring Harbor Laboratory



B. Stillman

The 74th Cold Spring Harbor Laboratory Symposium on Quantitative Biology on *Evolution: The Molecular Landscape* was dedicated to Charles Darwin on the occasion of the bicentennial of his birth and the 150th anniversary of the publication of *On the Origin of Species.* The Laboratory celebrated the 100th anniversary in 1959 with its 24th Symposium on *Genetics and Twentieth Century Darwinism.* What was entirely absent from that Symposium, and what dominated the Symposium 50 years later, are the contributions molecular biology has made to our understanding of evolution. Even as the details of Darwin's ideas have been modified over the years, evidence from molecular studies has strengthened his fundamental thesis.

The 2009 Symposium set out to examine the current state of many of the ideas that Darwin developed in his four great books: *On the Origin of Species by Means of Natural Selection, The Variation of Animals and Plants Under Domestication, The Descent of Man and Selection in Relation to Sex,* and *The Expression of Emotions in Man and Animals*. Leading investigators were invited to present their latest research in a diversity of fields ranging from the origins of life (unicellular and multicellular) to speciation and domestication to the evolutionary basis of human attributes. An overarching theme of the meeting was the extent to which much of evolutionary biology can now be viewed in a molecular, and often genomic, framework and the extraordinary degree to which many of Darwin's insights remain profoundly relevant today.

D. Stewart

The Symposium included two rather unusual sessions. Evolutionary concepts have had an impact far beyond the boundaries of science, and there is hardly a field of human endeavor that has not been influenced by evolutionary thinking. To acknowledge this contribution of Darwin, there was a session on "Cultural Evolution" that included presentations on principles of natural selection applied to linguistics, ideas, and economics by, respectively, Daniel Dennett, Matt Ridley, and Niall Ferguson. In the second unusual session, "Evolution and the Public," Kevin Padian, Ken Miller, Barbara Forrest, and Eugenie Scott discussed so-called "intelligent design" and the threat such irrational and antiscientific attitudes pose to education in the United States.

In arranging this Symposium, the organizers were dependent on the guidance of a broad cadre of advisors including Drs. Nicholas Barton, Hans Ellegren, Claire Fraser-Liggett, David Haussler, Gerry Joyce, Susan McCouch, Svante Pääbo, Sally Otto, Richard Wrangham, and James D. Watson. Opening night speakers included Janet Browne, Ed Wilson, David Kingsley, and Marc Hauser, and Douglas Wallace presented the Reginald Harris lecture on "Energetics in Eukarotic and Human Origins." Kevin Padian enlightened a mixed audience of scientists and lay friends and neighbors with his Dorcas Cummings Lecture on "Darwin, Dover, and Intelligent Design" and Brian Charlesworth ended the meeting with a masterful and throught-provoking summary.

This Symposium was attended by 400 scientists from over 25 countries, and the program included 69 invited presentations, six Symposium fellows short talks selected from the abstracts, and more than 175 poster presentations.

Funds to support 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.

PROGRAM

Introduction
B. Stillman, *Cold Spring Harbor Laboratory*

RNA and Proteins
Chairperson: S. Lindquist, *Whitehead Institute for Biomedical Research, Cambridge, Massachusetts*

Cellular Evolution
Chairperson: N. Patel, *University of California, Berkeley*

Selection and Adaptation
Chairperson: M. Bulyk, *Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts*

Cultural Evolution
Chairperson: L. Caporale, *New York*

Reginald B. Harris Lecture: Epigenetics in Eukaryotic and Human Origins
D.C. Wallace, University of California, Irvine

Diversity
Chairperson: H. Hoekstra, *Harvard University, Cambridge, Massachusetts*

Genome Evolution
Chairperson: C. Fraser-Liggett, *University of Maryland School of Medicine, Baltimore*

Evolution of Systems
Chairperson: R. Greenspan, *The Neurosciences Institute, San Diego, California*

Dorcas Cumming Lecture: Darwin, Dover, and Intelligent Design
K. Padian, *University of California, Berkeley*

Evolution and Development
Chairperson: N. King, *University of California, Berkeley*

Symposium Fellows
Chairperson: J. Thornton, *University of Oregon, Eugene*

Evolution and the Public
Chairperson: K. Padian, *University of California, Berkeley*

Domestication
Chairperson: D. Ware, *Cold Spring Harbor Laboratory*

Human Evolution
Chairperson: D. Page, *Whitehead Institute for Biomedical Research, Cambridge, Massachusetts*

Social Interactions
Chairperson: D. Page, *Whitehead Institute for Biomedical Research, Cambridge, Massachusetts*

Mutation
Chairperson: L. Shapiro, *Stanford University School of Medicine, California*

Summary
Chairperson: B. Charlesworth, *University of Edinburgh*

J. Witkowski, E.O. Wilson

V. Ramakrishnan, J. Szostak, A. Gann

MEETINGS

Plant Genomes: Genes, Networks, and Applications

March 4–7 111 Participants

ARRANGED BY **Steve Briggs,** University of California, San Diego
Mary Lou Guerinot, Dartmouth College
Detlef Weigel, Max-Planck Institute for Developmental Biology

The theme of the meeting highlighted the move from understanding the function of individual genes to the analysis of larger functional networks, and it highlighted ways in which this knowledge has increasingly practical applications. The latter is not limited to breeding, but it is also useful for ecological research. The expertise of the three coorganizers spanned the major areas of the meeting, from translational genomics (Mary Lou Guerinot and Steven Briggs) to natural genetic diversity (Detlef Weigel).

D. Weigel, M.L. Guerinot

Following the tradition of the meeting, the two keynote presentations, by Andrew Clark (Cornell) on comparative genomics in *Drosophila* and Aviv Regev (Broad institute/MIT) on regulatory network analysis in yeasts and mammals, provided exciting insights into current work outside of plants.

A special highlight of the meeting was the first session, celebrating the recently finished maize genome sequence. Virginia Walbot (Stanford) and Pat Schnable (Iowa State) demonstrated how maize geneticists are already benefitting from this achievement. Maize is also notable for being the first crop in which breeders and farmers made extensive use of the heterosis phenomenon, in which hybrid progeny outperforms its inbred parents. Loren Rieseberg (University of British Columbia), Dani Zamir (Hebrew University), Detlef Weigel, and Rob Martienssen presented very different approaches to studying and exploiting genetic and epigenetic effects in hybrids.

We are currently witnessing a revolution in DNA sequencing ability, as sequencing capacity at the same cost has been increasing severalfold annually over the past 3 years. Joe Ecker (Salk Institute), Brandon Gaut (University of California, Irvine), and Regine Kahmann (Max-Planck Institute for Terrestrial Microbiology) discussed excellent examples of the impact that these new technologies have on plant sciences.

Until recently, the major limitation in connecting genotype with phenotype was the acquisition of genotype information, be it by typing individual DNA markers or by sequencing complete genomes. With sequencing prices dropping rapidly, and no end in sight, this is completely changing, and the major bottleneck now is high-throughput phenotyping. Innovative approaches to this problem were showcased in the talks by Philip Benfey (Duke), Natasha Raikhel (University of California, Riverside), and Mary Lou Guerinot. Of course, the genotyping and phenotyping information needs to be captured and interpreted in an integrated fashion. Doreen Ware (CSHL), Jim Carrington (Oregon State), Lothar Willmitzer (Max-Planck Institute for Molecular Plant Physiology), and Tom Mitchell-Olds (Duke) demonstrated different approaches to solving this problem. The ultimate challenge then

P. Benfy, A Millar

is to combine these data and to construct genetic, cellular, and physical networks as discussed by Jan Traas (ENS Lyon), Elliot Meyerowitz (Caltech), Andrew Millar (Edinburgh University), and Gloria Coruzzi (New York University). The last area is the focus of the NSF-funded iPlant initiative, which was discussed following the network/models session in the context of presenting the results of a workshop that had been held at the Banbury Center under the auspices of NSF and several international funding agencies.

As mentioned above, not only are the advances in plant biology impacting plant breeding, but they are also important for understanding evolutionary and ecological processes in general, as was discussed by Joy Bergelson (University of Chicago) and Masa Yano (NIAS Tsukuba). Finally, in addition to the invited presenters mentioned by name, each session featured shorter talks drawn from submitted abstracts. Those not selected had a chance to present their work in a poster session.

Although the meeting was smaller than in previous years, attendees were all very happy about the high caliber of talks and many mentioned that the speaker lists had made this program particularly attractive. Apart from the current funding climate allowing fewer scientists to attend conferences, this conference was held between the Plant and Animal Genome Meeting in San Diego in January and the Annual Maize Meeting the following week, and these were likely factors that reduced the number of potential attendees.

This meeting was supported by the National Science Foundation and a gift from Dow Agrosciences.

PROGRAM

Keynote Address: The *Drosophila* 12 Genomes Project
A. Clark, *Cornell University*

Celebrating the Maize Genome
Chairperson: V. Walbot, *Stanford University, California*

Hybrids and Heterosis
Chairperson: L. Rieseberg, *University of British Columbia, Vancouver, Canada*

Genomes: Plants And Microbes
Chairperson: J. Ecker, *Salk Institute for Biological Studies, La Jolla, California*

Innovative Phenotyping
Chairperson: P. Benfey, *Duke University, Durham, North Carolina*

Integrating Omics
Chairperson: D. Ware, *Cold Spring Harbor Laboratory*

Models/Iplant/*Arabidopsis* 2010
Chairperson: J. Traas, *Ecole Normale Supérieure de Lyon, France*

Discussion of *Arabidopsis* 2010 and Plant Genome Research Program
Led by G. Coruzzi, A. Millar, and J. Ecker

Keynote Address: Transcriptional Network Evolution
A. Regev, *Broad Institute*

Evolution Inside and Outside the Lab
Chairperson: J. Bergelson, *University of Chicago, Illinois*

D. Ware, A. Eveland

K. Slotkin, M. Katari

Systems Biology: Networks

March 18–22 177 Participants

ARRANGED BY **Anne-Claude Gavin,** EMBL, Germany
Trey Ideker, University of California, San Diego
Marc Vidal, Dana-Farber Institute/Harvard Medical School
Marian Walhout, University of Massachusetts Medical School

Central to Systems Biology is the notion that all living systems are made up of networks of interactions. These networks span multiple scales of space and time, from networks of interacting atoms that influence protein structure, to networks of genes and proteins, to networks giving rise to disease. Biological networks also come in a variety of flavors, including networks related to transcription, posttranscription, translation, physical interaction, genetic interaction, metabolic flow, and functional relationships. This year's program focused on progress related to several of these major types: (1) genetic, (2) transcriptional, (3) metabolic, (4) signaling, (5) protein–protein, and (6) posttranscriptional. The program opened on Wednesday with the first of two Keynote Addresses from Eric Davidson on global gene regulatory networks in sea urchin development. The goal of Davidson's research is a global network in which all inputs derive from nodes within the network, obtained from a model of the genomic regulatory code directing embryonic specification. The opening day continued with an evening session on genetic networks, with discussions ranging from linkage analysis to interactions and pathways. The program continued on Thursday with a day filled with two sessions devoted to transcriptional networks. Late afternoon on Thursday welcomed all attendees to enjoy a wine and cheese party. Accepted posters were divided into two groups and presented over two poster sessions on Thursday and Saturday. Friday included three important sessions on network medicine, metabolic networks, and protein–protein interactions. The program continued on Saturday with a session on signaling. Saturday evening provided the attendees with a second well-received Keynote Address from James Ferrell, concluding the evening with a banquet of cocktails and dinner. The full program concluded on Sunday with a session devoted to posttranscriptional networks.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.

PROGRAM

Genetic Networks
Chairperson: A.G. Fraser, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Transcriptional Networks I
Chairperson: M. Walhout, *University of Massachusetts Medical School, Worcester*

Transcriptional Networks II
Chairperson: M. Bulyk, *Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts*

Network Medicine
Chairperson: T. Ideker, *University of California, San Diego*

Metabolic Networks
Chairperson: J. Collins, *Boston University, Massachusetts*

Keynote Address: Toward a Global Gene Regulatory Network for Sea Urchin Development
E. Davidson, *California Institute of Technology*

Protein–Protein Interactions
Chairperson: A.-C. Gavin, *European Molecular Biology Laboratory, Heidelberg, Germany*

Signaling
Chairperson: S. Gaudet, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

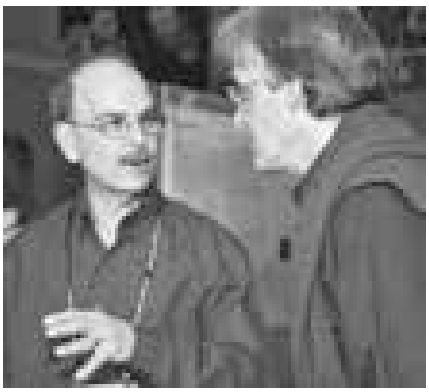
Keynote Address
J. Ferrell, *Stanford University School of Medicine*

Posttranscriptional Networks
Chairperson: F. Piano, *New York University, New York*

Computational Cell Biology

March 24–27 123 Participants

ARRANGED BY **Galit Lahav,** Harvard Medical School
Leslie Loew, University of Connecticut Health Center
Alex Mogilner, University of California, Davis
John Tyson, Virginia Polytechnic Institute & State University



L. Loew, J. Tyson

The inventory of molecules participating in pathways and generating forces that regulate many aspects of cell physiology is now known in great detail. However, these pathways and mechanochemical networks are so complex as to defy reliable understanding by an intuitive, qualitative reasoning. During the past decade, it has become clear that computer simulations based on realistic biophysical models of regulatory networks can be useful in making sense of enormous volumes of quantitative data and in guiding new experimental studies. Success in this endeavor requires close collaboration among life scientists, mathematical modelers, and computer scientists. In March 2001, Les Loew and his colleagues at the University of Connecticut Health Center hosted the first multidisciplinary workshop on Computational Cell Biology at the Cranwell Center in Lenox, Massachusetts. The meeting was attended by an enthusiastic group of quantitative biochemists and cell biologists, modeling experts, and software developers. The first meeting featured oral presentations and poster sessions that highlighted the interplay between experiments and models, and a unique afternoon session of software demonstrations. By popular demand, the UConn group hosted two more workshops, in 2003 and 2005. At the third meeting, a leadership group was commissioned to find a more permanent home for the meeting, with Cold Spring Harbor Laboratory identified as the first place to try. When approached with this idea, David Stewart immediately gave his full support and scheduled the first Computational Cell Biology Meeting at Cold Spring Harbor in March 2007. The 2007 meeting was organized by John Tyson, Les Loew, and Tom Pollard, assisted by a distinguished panel of session chairs.

In 2009, the fifth (second at CSHL) meeting returned, and the format of the first CSHL meeting was maintained, including the popular software demos, along with an emphasis on the most mature areas of the field (neuronal cell biology, cell signaling, cytoskeletal dynamics, development, cell growth, division and death). Compared to the previous meeting, a session on bacterial physiology was not organized in order to decrease an overlap with more systems-biology-oriented meetings. In addition, the organizers decided that there is no need to invite session organizers—four meeting organizers can effectively play this role themselves.

The oral presentations at the meeting consisted of longer invited talks by established leaders in the field and of shorter talks chosen from abstracts submitted by registered participants; similar to the previous meeting, this combination was very successful. This way, many young graduate students and postdocs were given a chance to showcase their work before an interdisciplinary audience, and they were excellent in illustrating the power of a combined experimental-theoretical attack on outstanding problems in cell biology. The quality of the poster session was also very high; this session was lively and judging from remarks of many participants very enjoyable. One problem with scheduling that is becoming clear from the last two meetings is that the schedule is very tight due to the fact that the meeting structure was inherited from the Lenox workshops, and the duration shortened. Perhaps one fewer session, or fewer talks per session, or just one Keynote Address has to be planned for the future.

U. Alon

The meeting was opened by a Keynote Address by Uri Alon, who gave a chalk talk on robustness in cell regulatory networks. The second Keynote Address was given by Ravi Iyengar, who told about recent work of his lab on deciphering cell signal transduction. Cell signaling (two sessions) continued to be the leading theme of the meeting with exciting talks by J. Gunawardena (Harvard) about multiple steady state in dynamics of posttranslational modification codes, Tim Elston (University of North Carolina) on modeling signaling events that lead to chemotrophic growth, A. van Oudenaarden (Massachusetts Institute of Technology) on stochastic effects in cell "decision making," Long Cai (California Institute of Technology) on stochastic frequency modulation in calcium localization bursts, and a number of short talks. The neuronal biology session was met with significant enthusiasm opened by fascinating reports on information processing by Samuel Wang (Princeton), multiscale modeling of the brain by U. Bhala (NCBS, Bangalore), and the neocortex as a distributed network of linear integrators by R. Yuste (Columbia), again followed by excellent short talks. The cytoskeletal dynamics session vibrated with excitement created by O. Weiner's (Unversty of California, San Francisco) report on pioneering experiments promising to start a next generation of quantitative experimentation in cell biology and on waves in chemotactic and motile cells. This was followed by equally excellent talk by R. Loughlin (University of California, Berkeley), still a graduate student, on a combination of modeling and experiment promising to unravel the mechanism of mitotic spindle maintenance. The cell growth, division, and death session was opened by eminent P. Sorger (Harvard), who told about using systems biology methods to understand cell-to-cell variability in response to death ligands. A. Anderson/V. Quaranta (Florida University/Vanderbilt) followed with the talk on developing and verifying a model of tumor invasion. J. Toetcher/ A. Loewer (Massachusetts Institute of Technology/Harvard) introduced the audience to the mammalian DNA-damage response mechanisms. The development session was sparkling with brilliance of two invited talks by Vic Foe (Washington University) on using agent-based modeling and experiments to prove that multiple redundant mechanisms are responsible for mitotic apparatus regulating cytokinesis, and by Steve Altschuler (University of Texas) on heterogeneity in cancer cells and on the role of discreteness in yeast polarization. Finally, a very special session on new methods and software was started by J. Stiles (Pittsburgh) who reported advances in three-dimensional in silico multiscale simulations of cell molecular processes. His talk was followed by a number of short reports and many demonstrations.

A special feature of this meeting was an informal talk by Uri Alon on how to nurture young scientists.

The uniformly high quality of the science reported at this meeting and the cooperative spirit of the experimentalists, theoreticians, and computer scientists in attendance upheld the opinion of the Cold Spring Harbor Meetings staff to welcome Computational Cell Biology into their regular meeting schedule. The meeting will continue on its biennial schedule (March/April 2011). Quantitative measurements and realistic mathematical modeling are here to stay in cell biology, and this CSHL meeting promises to be a flagship venue for reporting the best developing work in the field.

The meeting was well supported by a grant from the National Institutes of Health, through the National Technology Center on Networks and Pathways at the University of Connecticut Health Center. This grant will be most likely gone and unavailable to support one more similar meeting; more money will have to be applied for (perhaps to NSF who indicated an enthusiasm for this meeting series).

PROGRAM

Keynote Address
U. Alon, *Weizmann Institute*

Cell Signaling I
Chairperson: J. Tyson, *Virginia Polytechnic Institute and State University, Blacksburg*

Neuronal Cell Biology
Chairperson: L. Loew, *University of Connecticut Health Center, Farmington*

Poster Session

Keynote Address: Cell State Change Decisions Triggered by Receptors
Chairperson: R. Iyengar, *Mount Sinai School of Medicine*

Cytoskeletal Dynamics
Chairperson: A. Mogilner, *University of California, Davis*

Cell Growth, Division, and Death
Chairperson: G. Lahav, *Harvard Medical School, Boston, Massachusetts*

New Tools and Software Development
Chairperson: I.I. Moraru, *University of Connecticut Health Center, Farmington*

Development
Chairperson: A. Mogilner, *University of California, Davis*

Cell Signaling II
Chairperson: J. Tyson, *Virginia Polytechnic Institute and State University, Blacksburg*

A. Mogilner, T. Elston

S. Altschuler, G. Lahav

Carticaturist at the cocktail party

Molecular and Cellular Biology of Plasminogen Activation

March 31–April 4 113 Participants

ARRANGED BY **Katherine Hajjar,** Weill Cornell Medical College
Stella Tsirka, University Medical Center at Stony Brook
Elaine Wolff, University of Illinois at Urbana, Champaign

S.K. Hajjar, S. Tsirka

This 12th Workshop is unique in that the scientific program is entirely abstract-driven. Thus, all of the presentations are selected from submitted abstracts. This allows emphasis upon new discoveries that are largely unpublished, and fosters productive interactions among young investigators, postdoctoral fellows, graduate students, and established investigators. Key subject areas of the meeting included structure, function, and regulation of the plasminogen activator system, matrix metalloproteinases and novel proteases; plasminogen activators in inflammation and host defense, tumor biology, neurobiology, tissue remodeling, and novel therapeutics; and vascular biology and angiogenesis.

The meeting was subscribed to by 108 attendees and included participants from 15 countries including Australia, Austria, Belgium, Canada, Denmark, France, Germany, Italy, Japan, Mexico, Norway, Spain, Sweden, United Kingdom, and the United States. The attendees included 48 students and postdocs (44%), meeting one of the objectives of encouraging participation by young investigators in the workshop. Not only did young investigators participate in the workshop, but about 75% of the presentations were given by young investigators. Almost half of the participants were women, with five of them serving as session chairs.

PROGRAM

Keynote Address: Modeling, Mimicking, and Modulating Tumor Metastasis: Involvement of a Few Old (Protease) Friends
J. Quigley, *Scripps Research Institute*

Structure, Function, and Regulation of the Plasminogen Activator System
Chairpersons: N. Behrendt, *Finsen Laboratory, Copenagen, Denmark;* K. Akassoglou, *Gladstone Institutes, University of California, San Francisco*

Plasminogen Activators, Matrix Metalloproteinases, and Novel Proteases
Chairpersons: S. Netzel-Arnett, *University Of Maryland, Baltimore;* H. Crawford, *SUNY, Stony Brook University*

Plasminogen Activators in Inflammation and Host Defense
Chairpersons: P. Ragno, *University of Salerno, Italy;* T. Bugge, *National Institutes of Health, Bethesda, Maryland*

Vascular Biology and Angiogenesis
Chairpersons: D. Falcone, *Weill Medical College of Cornell University, New York;* S. Gonias, *University of California School of Medicine, San Diego, La Jolla*

Plasminogen Activators and Tumor Biology
Chairpersons: R. Szabo, NIDCR, *National Institutes of Health, Bethesda, Maryland;* F. Blasi, *IFOM-FIRC Institute of Molecular Oncology, Milano, Italy*

Plasminogen Activators and Neurobiology
Chairpersons: G. Hoyer-Hansen, *Finsen Laboratory-Rigshospitalet, Copenhagen, Denmark;* D. Lawrence, *University of Michigan, Ann Arbor*

The Plasminogen Activator System and Tissue Remodeling
Chairpersons: A. Deora, *Weill Medical College of Cornell University, New York;* S. Strickland, *The Rockefeller University, New York*

The Plasminogen Activator System and Novel Therapeutics
Chairpersons: R. Medcalf, *Monash University, Victoria, Australia;* P. Declerck, *University of Leuven, Belgium*

Synapses: From Molecules to Circuits and Behavior

April 14–18 200 Participants

ARRANGED BY **Hollis Cline,** Cold Spring Harbor Laboratory
Richard L. Huganir, Johns Hopkins University School of Medicine/HHMI
Thomas Sudhof, University of Texas Southwestern Medical Center

T. Grodzicker, H. Cline

The brain consists of a vast network of excitable cells (neurons) that conduct electrical impulses and communicate with each other via specialized junctions (synapses). Information is processed and stored in the nervous system through patterns of electrical activity and via changes in the strength and structure of synapses. All aspects of nervous system function, including perception, cognition, and action, depend on proper information processing by synapses. As master regulators of neuronal excitability and synaptic communication, ion channels and receptors lie at the heart of neurobiology. In recent years, the molecular and cell biological analysis of neuronal ion channels and receptors has revolutionized our understanding of the basic mechanisms that control electrical signaling and synaptic function in the nervous systems. The convergence of advances in biochemistry, molecular genetics, microscopic imaging, and electrophysiology has made synaptic biology one of the most exciting and rapidly growing in neuroscience. Increasingly, scientists are moving to in vivo systems to investigate the synaptic basis of behavior in living animals via the genetic engineering of channels, receptors, and other synaptic proteins in mice and other model organisms.

The entire field is poised for further breakthroughs that will not only illuminate basic workings of the brain, but also shed light on neurological and psychiatric diseases that stem from abnormal neuronal excitability and synaptic dysfunction. Indeed, genetic association studies already point to genes for synaptic structure and function as being involved in neuropsychiatric illnesses, in particular autism. Future advances will be facilitated by cross-fertilization of ideas and technologies between scientists studying channels, receptors, and synapses at all levels, in diverse organisms and using different methodological approaches.

M. Zhen, T. Sudhof

This fourth meeting was very successful, bringing together participants from the United States, Europe, and Asia in an atmosphere of social and scientific exchange. As listed below, a wide range of topics were discussed. Compared to earlier meetings, the 2009 meeting emphasized the physiology and pathophysiology of synapses and the behavioral and systems implications of their functions. More than 50 attendees were selected to give oral presentations of their work, and the majority of the rest presented posters. Outstanding plenary lectures were given by Clay Ried and Cori Bargmann.

The overall response to the meeting was extremely positive, continuing to cement the reputation of this Cold Spring Harbor conference in the field. Attendees enjoyed the breadth of subjects covered and the chance to interact with investigators in related but distinct fields. The unusual opportunity for junior investigators (including postdocs and students) to present their own work was welcomed.

The meeting was supported with funds from the National Institute on Drug Abuse and the National Institute of Mental Health, branches of the National Institutes of Health.

PROGRAM

Keynote Address: Toward Complete Functional and Structural Imaging of Cortical Circuits
R.C. Reid, *Harvard Medical School*

Diseases of Synapses and Circuits
Chairpersons: U. Heberlein, *University of California, San Francisco;* C. Walsh, *Beth Israel Deaconess Medical Center, Boston, Massachusetts*

Ion Channels, Receptors, and Transporters
Chairpersons: A. Patapoutian, *Scripps Research Institute, Del Mar, California;* E. Gouaux, *Oregon Health and Science University, Portland*

Synaptic Formation and Function
Chairpersons: Y. Jin, *University of California, San Diego;* H. McMahon, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

Synaptic Plasticity
Chairpersons: P. Castillo, *Albert Einstein College of Medicine, Bronx, New York;* L. Chen, *University of California, Berkeley*

Imaging Synaptic Structure and Function
Chairpersons: R. Yasuda, *Duke University Medical Center, Durham, North Carolina;* G. Miesenboeck, *Oxford University, United Kingdom*

Imaging Neuronal Circuits
Chairpersons: A.-S. Chiang, *National Tsing Hua University, Hsinchu, Taiwan;* J. Fetcho, *Cornell University, Ithaca, New York*

Neuronal and Circuit Plasticity
Chairpersons: L. Luo, *Stanford University, California;* F. Engert, *Harvard University, Cambridge, Massachusetts*

Keynote Address: A Hub-and-Spoke Circuit for Pheromone Attraction and Social Behavior in *C. elegans*
C. Bargmann, The Rockefeller University

Behavioral Plasticity
Chairpersons: D. Anderson, *California Institute of Technology, Pasadena;* J. Tsien, *Medical College of Georgia, Augusta*

R. Huganir, C. Bargmann

Poster session in Bush Auditorium

The Ubiquitin Family

April 21–25 225 Participants

ARRANGED BY **Judith Frydman,** Stanford University
J. Wade Harper, Harvard Medical School
Matthias Peter, Institute of Biochemistry, Switzerland
Brenda Schulman, St. Jude Children's Research Hospital

B. Schulman, J. Frydman, J.W. Harper

This fourth meeting, following the successful inauguration of this series in 2003, focused on ubiquitin and a group of structurally related "ubiquitin-like" proteins, and their roles in regulation of various cellular processes. Major questions in the field include how specificity in ubiquitin and ubiquitin-like protein conjugation reactions is maintained, and the molecular mechanisms that are used to control the specificity of ubiquitin chain linkages. These questions are being elegantly addressed using structural biology, sophisticated kinetic studies, systematic library screening, and proteomics technologies by an increasing number of investigators in the field. In addition, important advances continue to be made in understanding how ubiquitin and its family members contribute to the operation of diverse cellular pathways, and how these functions are perturbed in diseases ranging from viral infections to cancers. Exciting progress is also being made in the development of small-molecule inhibitors of a range of enzymes involved in ubiquitin and ubiquitin-like protein pathways.

The meeting this year attracted more than 220 scientists who engaged in lively discussions concerning the roles of ubiquitin in protein turnover, the structure and mechanism of a large multisubunit protease called the proteasome that catalyzes the degradation of ubiquitinated proteins, and the involvement of ubiquitin quality-control mechanisms used by cells to eliminate otherwise toxic misfolded proteins. There is also a growing understanding of nonproteolytic roles of ubiquitin and ubiquitin-like proteins. A wide array of experimental systems, including yeast, plants, and mammals, were used to probe the function of ubiquitin and ubiquitin-like proteins in diverse physiological processes ranging from control of mitosis, hormone signaling in plants, metabolic control of sulfur

A. Goldberg, D. Bar-Sagi

R. Sever, D. Nath

incorporation into tRNA, to how the proteasome is assembled. Other highlights of the meeting this year concerned progress on the dynamics of enzymes that function in ubiquitin conjugation, revealing how in-depth kinetic analyses of these systems can reveal underlying mechanisms controlling the rate and specificity of substrate ubiquitination. In summary, this meeting has continued to be a unifying forum that helps us understand the regulatory mechanisms by which ubiquitin and ubiquitin-like proteins function and their ever-growing roles in cellular physiology and disease.

The meeting was funded by the National Cancer Institute and the National Institute of Child Health & Human Development, branches of the National Institutes of Health.

PROGRAM

Conjugation, Removal, and Recognition of Ubiquitin and Ubiquitin-like Proteins I
Chairpersons: N. Zheng, *University of Washington, Seattle;* K.D. Wilkinson, *Emory University School of Medicine, Atlanta, Georgia*

Conjugation, Removal, and Recognition of Ubiquitin and Ubiquitin-like Proteins II
Chairpersons: R. Klevit, *University of Washington, Seattle;* M. Hochstrasser, *Yale University, New Haven, Connecticut*

Substrate Targeting and Degradation
Chairpersons: A. Goldberg, *Harvard Medical School, Boston, Massachusetts;* M. Tyers, *Mount Sinai Hospital, Toronto, Canada*

Nuclear Functions of UBLs
Chairpersons: K. Vousden, *Beatson Institute for Cancer Research, Glasgow, United Kingdom;* J.-M. Peters, *Research Institute of Molecular Pathology, Vienna, Austria*

Regulation of Intracellular Pathways
Chairpersons: D. Finley, *Harvard Medical School, Boston, Massachusetts;* R. Deshaies, *California Institute of Technology, Pasadena*

Quality Control
Chairpersons: R. Hampton, *University of California, San Diego;* T. Sommer, *Max-Delbruck Center for Molecular Medicine, Berlin, Germany*

UBLs in Signaling I: Receptors, Plasma Membrane/Endosomal Trafficking
Chairpersons: D. Rotin, *Hospital for Sick Children, Toronto, Canada;* A. Weissman, *NCI-Frederick, Maryland*

UBLs in Signaling II: Intracellular Communication and Regulation of Organismal Biology
Chairpersons: D. Bar-Sagi, *New York University Medical Center, New York;* F. Melchior, *University of Göttingen, Germany*

Spring shower!

Poster session in Grace lobby

M. Hochstrasser, N. Fang

Telomeres and Telomerases

April 28–May 2 314 Participants

ARRANGED BY **Joachim Lingner,** Swiss Institute for Experimental Cancer Research
Virginia Zakian, Princeton University
Dorothy Shippen, Texas A&M University

L. Vega, V. Zakian

The conference consisted of eight sessions of talks and two poster sessions. As in 1999, 2001, 2003, and 2005, the format was to invite two chairs per session, who were a mix of established scientists in the field and younger scientists who had already made their mark by publishing as independent investigators. Many session chairs gave a scientific (12 min) presentation. The rest of the presentations (also 12 min) were chosen from submitted abstracts, allowing as many presentations as possible. These presentations were primarily by graduate students and postdoctoral fellows. Attendance exceeded 300 participants, a high fraction of whom presented the 150 posters and 80 talks.

The talks and posters covered all aspects of telomere and telomerase biology, including telomerase structure, enzymology, and regulation, telomere length regulation, protection and processing of chromosome ends, the consequences of telomere dysfunction, telomere dynamics in cancer, and telomerase-independent telomere maintenance.

The scientific content was very high throughout the conference in both the talks and the posters. A large body of unpublished data was presented and extensively discussed in an open fashion. Formal and informal discussions were lively and informative. The conference was judged to be highly successful based on verbal and email communications to the organizers. There is strong enthusiasm for another meeting on the same topic in 2011.

The meeting was funded by the National Institute on Aging, a branch of the National Institutes of Health.

PROGRAM

Telomerase Structure and Biochemistry
Chairpersons: T. Cech, *University of Colorado, Boulder;* Y. Tzfati, *Hebrew University of Jerusalem, Israel*

End Resection and Protection
Chairpersons: C. Price, *University of Cincinnati, Ohio;* A. Bertuch, *Baylor College of Medicine, Houston, Texas*

Telomeres and DNA Damage Sensing
Chairpersons: S. Gasser, *Friedrich Miescher Institute, Basel, Switzerland;* C. Greider, *Johns Hopkins University, Baltimore, Maryland*

Telomere Length Regulation
Chairpersons: L. Harrington, *Ontario Cancer Institute, Toronto, Canada;* K. Friedman, *Vanderbilt University, Nashville, Tennessee*

Consequences of Telomere Dysfunction
Chairpersons: S. Marcand, *CEA, Fontenay-aux-Roses, France;* E. Blackburn, *University of California, San Francisco*

Proteins and RNA Bound to Telomeric and Subtelomeric Chromatin
Chairpersons: J. Karlseder, *The Salk Institute, La Jolla, California;* Z. Songyang, *Baylor College of Medicine, Houston, Texas*

Telomeres in Senescence, Proliferation, and Cancer
Chairpersons: M. Blasco, *Spanish National Cancer Centre, Madrid;* W. Wright, *University of Texas Southwestern Medical Center, Dallas*

Telomeres and Recombination
Chairpersons: R. Wellinger, *Université de Sherbrooke, Canada;* K. Riha, *Austrian Academy of Sciences, Vienna*

The Bovine Genome

May 9–11 54 Participants

ARRANGED BY **Christine Elsik,** Georgetown University
Harris Lewin, University of Illinois at Urbana Champaign
Jerry Taylor, University of Missouri
Kim Worley, Baylor College of Medicine

This special meeting was hosted by the Bovine Genome Sequencing and Analysis Consortium and the Bovine HapMap Consortium to present the state of the art in bovine genome sequencing, assembly, analysis, and applications to the animal agriculture constituency; to discuss future research priorities based on the consortia's accomplishments; and to promote bovine as a model for mammalian biology and evolution. The meeting was attended by scientists from 13 countries and included university faculty, postdoctoral fellows, and graduate students; government research scientists; and cattle industry representatives.

Invited presentations were made by 15 scientists representing the Sequencing and HapMap Consortia and 22 poster abstracts were presented. A number of the poster presentations were made by graduate students and postdoctoral fellows attending the meeting. The meeting was warmly received by all participants, with the hope that further opportunity will arise to support the attendance of many younger scientists at future meetings.

The organizers are grateful for the sponsorship of Affymetrix, Illumina, Roche/454, Merial, Pfizer, the USDA ARS, and CSREES. Without this assistance, the meeting would not have been possible.

PROGRAM

Plenary Session
Chairperson: C. Elsik, *Georgetown University, Washington, D.C.*

Evolution and Comparative Genomics
Chairperson: R. Tellam, *CSIRO, St. Lucia, Australia*

From Genome to Biology
Chairperson: M. Rijnkels, *Baylor College of Medicine, Houston, Texas*

Discussion Panel: Next Generation of Tools and Resources
Panel Leader: S. Kappes, *USDA-ARS, Beltsville, Maryland*
Panel Members: S. Bauck, *Merial*
M. Allan, *Pfizer Animal Genetics*
T. Harkins, *Roche 454*
R. Barkovich, *Affymetrix*
D. Bailey, *Illumina*
D. Hamernick, *University of Nebraska, Lincoln*

Genomic Variation and Population Genetics
Chairperson: W. Barendse, *CSIRO, St. Lucia, Australia*

Genomic Technologies and Applications
Chairperson: C. Van Tassell, *USDA-ARS, Beltsville, Maryland*

The Biology of Genomes

May 5–9 537 Participants

ARRANGED BY **Michael Ashburner,** University of Cambridge
Andrew Clark, Cornell University
Kerstin Lindblad-Toh, Broad Institute
George Weinstock, Washington University

This annual meeting marked the 22nd annual gathering of genome scientists in this setting. The past decade has seen remarkable progress in the mapping, sequencing, and annotation of the genomes of many "model organisms" and publication of finished and draft sequences of human genomes, in addition to model organisms and hundreds of bacteria. Most remarkable has been the enormous progress in DNA sequencing technology over the last few years, leading to advances in many areas with notable breakthroughs in human applications, particularly in cancer genomics. Just over 500 people from around the world attended the meeting, with more than 300 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

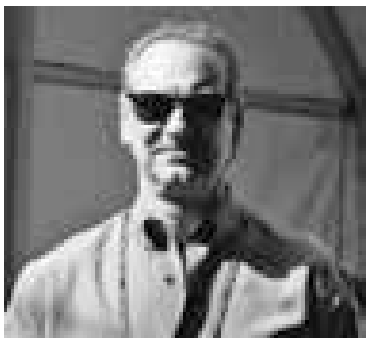
G. Weinstock, H. Sussman, A. Clark

The session topics are listed below. There were numerous reports on progress in using new sequencing technologies, with high expectations for additional advances as these become more widely available. The keynote talks were delivered by Claire Fraser-Liggett and Barbara Meyer.

The ELSI (Ethical, Legal, and Social Implications) panel was chaired by Laura Rodriguez and the discussion topic was Data Sharing, Identifiably, and Informed Consent in Community Resource Genomics Projects.

Major sponsorship for this meeting meeting was provided by Roche. Additional funding was provided by Illumina, Inc., and the National Human Genome Research Institute, a branch of the National Institutes of Health.

T. Gingeras



R. Wilson

T. Hubbard

PROGRAM

Functional and Cancer Genomics
Chairpersons: L. Chin, *Harvard Medical School, Boston, Massachusetts;* V. Velculescu, *Johns Hopkins University, Baltimore, Maryland*

Genetics of Complex Traits
Chairpersons: T. Mackay, *North Carolina State University, Raleigh;* J. Cohen, *University of Texas Southwestern Medical Center, Dallas*

High-throughput Genomics and Genetics
Chairpersons: B. Wold, *California Institute of Technology, Pasadena;* J. Ecker, *Salk Institute for Biological Studies, La Jolla, California*

Genetics and Genomics of Nonhuman Species
Chairpersons: S. Dellaporta, *Yale University, New Haven, Connecticut;* J. Werren, *University of Rochester, New York*

ELSI Panel Discussion: Data Sharing, Identifiability, and Informed Consent in Community Resource Genomics Projects
Chairpersons: L.L. Rodriguez, *National Human Genome Research Institute, NIH*
S. Wallace, *University of Montreal*
K. Kato, *Kyoto University*
B. Koenig, *Mayo Clinic*
A. Cambon-Thomsen, *University of Toulouse*

Evolutionary Genomics
Chairpersons: C. Ponting, *University of Oxford, United Kingdom;* A. Siepel, *Cornell University, Ithaca, New York*

Between sessions

Population Genomic Variation
Chairpersons: F. DiPalma, *Broad Institute, Cambridge, Massachusetts;* G. McVean, *University of Oxford, United Kingdom*

Guest Speakers
C. Fraser-Liggett, *University of Maryland School of Medicine, Baltimore*
B. Meyer, *HHMI/University of California, Berkeley*

Computational Genomics
Chairpersons: A. Regev, *Broad Institute, Cambridge, Massachusetts;* O. White, *University of Maryland, Baltimore*

E. Pennisi

D. Schwartz

Phosphorylation, Signaling, and Disease

May 12–16 167 Participants

ARRANGED BY **Sara Courtneidge,** The Burnham Institute
Ben Neel, Ontario Cancer Institute
Nicholas Tonks, Cold Spring Harbor Laboratory

This eighth meeting brought together scientists from the United States, Europe, the Far East, and South Pacific. Specifically focused on tyrosine phosphorylation in prior years, the meeting was expanded this year to include the most current work on the structure, regulation, and function of all protein kinases and protein phosphatases in biology. The meeting began with an opening keynote address by Benjamin Cravatt.

The format of the meeting was designed around areas of biological interest, rather than according to particular enzymes or enzyme families. A major thrust within the pharmaceutical industry is to exploit signal transduction pathways as sources of targets for novel therapeutic strategies. Therefore, the program for this meeting addressed the role of protein phosphorylation in the regulation of signal transduction under normal and pathophysiological conditions.

There were 54 speakers selected to present their data in sessions that dealt with receptor-proximal signaling; physiology and disease; cancer; metabolic and stress signaling; signaling pathways in survival and proliferation, and model systems. A variety of systems were described with great progress reported in genetic and biochemical approaches to the characterization of physiological functions for protein phosphorylation.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health. and with contributions from the Corporate Sponsors.

B. Neel, E. Miraldi

E. Miraldi, F. Chaudhary

PROGRAM

Keynote Address
B. Cravatt, *The Scripps Research Institute*

Receptor Signaling
Chairperson: S. Muthuswamy, *Ontario Cancer Institute, Toronto, Canada*

Cancer
Chairperson: T. Van Dyke, *NCI-Frederick, Maryland*

Metabolism
Chairperson: T. Tiganis, *Monash University, Melbourne, Australia*

Nonreceptor Kinases
Chairperson: R. Marais, *Institute of Cancer Research, London, United Kingdom*

Development, Disease, and Darwin
Chairperson: A. Rao, *Harvard Medical School, Boston, Massachusetts*

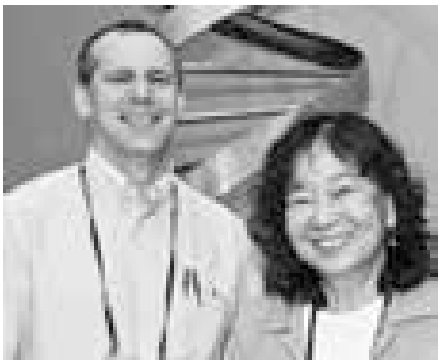
Downstream Signaling
Chairperson: L. Feig, *Tufts University School of Medicine, Boston, Massachusetts*

Tumor Suppressors
Chairperson: M. Park, *McGill University Cancer Center, Montreal, Canada*

Retroviruses

May 18–23 491 Participants

ARRANGED BY **Alan Engelman,** Dana-Farber Cancer Institute
Wei-Shau Hu, National Cancer Institute



A. Engelman, W.-S. Hu

This conference focused on recent progress in retrovirology, including studies of human pathogens such as human immunodeficiency virus 1 (HIV-1) and human T-lymphotropic virus I (HTLV-1), animal retroviruses such as Rous sarcoma virus (RSV) and feline immunodeficiency virus (FIV), and endogenous elements such as endogenous retroviruses and yeast transposable elements. A majority of the submitted abstracts focused on studies of virus replication mechanisms and host cell–virus interactions. Additionally, studies on antiviral compounds, drug-resistance mechanisms, potential new human pathogens, and results from clinical trials were presented.

Following the recent format of the meeting, two keynote speakers were invited to each present a 1-hour talk and all of the other presentations were selected by the organizers from submitted abstracts. The invited keynote speakers were Dr. Ronald Desrosiers and Dr. Volker Vogt. Dr. Desrosiers is a Professor at Harvard University and the Director of New England Primate Research Center. He is a highly respected pioneer in simian immunodeficiency virus (SIV) animal models for HIV-1 studies and vaccine research. He presented a historic progression of SIV studies in vaccine development and pathogenesis, discussing the discovery of Nef, an accessory protein, and its essential role in maintaining viral pathogenicity in macaques and in humans. Additionally, Dr. Desrosiers presented a recent study on a promising novel approach for vaccine development, which stimulated significant discussion that extended beyond the confines of Grace Auditorium. Dr. Volker Vogt, a professor from Cornell University, has made tremendous contributions to our current understanding of retrovirus assembly. Dr. Vogt first gave a historic perspective of the retrovirus field and the Cold Spring Harbor retroviruses (RNA Tumor Virus) meetings. He then centered his talk on his journey in scientific research and the lessons he learned. In the last 5 minutes, he stepped out of his scientific

M. Thali, E. Freed

T. Hope, K. Beemon, C.M. Stoltzfux

work and talked about who we are as scientists and his view of living without bias in other aspects of our lives to make our world a better place. Dr. Vogt's talk was poignant and earnest. Many scientists said that Dr. Vogt's talk was the most inspiring and moving keynote talk in the history of the Retroviruses meeting.

Poster melee

The submitted abstracts were organized into 10 oral presentation sessions and three poster presentation sessions. The 10 oral sessions are listed below. One of the most high-profile topics of the meeting was the function of the host protein Tetherin/Bst-2 and the mechanisms different viruses use to counter this host restriction factor. Tetherin was discovered in 2008 by the laboratories of Drs. Paul Bieniasz and John Guatelli as a host factor that inhibits HIV-1 infection by blocking the release of virus from infected cells. These seminal studies moreover identified the HIV-1 accessory protein Vpu as the viral countermeasure of tetherin action. More than a dozen talks from various laboratories were given in the 2009 Retroviruses meeting on the recent progress in our understanding of this topic. Tetherin inhibits the replication of multiple retroviruses as well as other enveloped viruses like those belonging to Filoviridae (Ebola and Marburg virus). Various viruses have interestingly evolved different mechanisms to counter tetherin's action. Although HIV-1 uses Vpu to suppress tetherin function, various SIVs, including SIV_{mac}, SIV_{agm}, SIV_{gsn}, and SIV_{rcm}, use Nef to achieve the same goal. As expected from studies of other restriction factors, the interplay between different animal tetherins and viruses is species-specific. The species-specific feature may provide an explanation to the intriguing observation that SIV_{mac} and HIV-2, two very closely related viruses that recently diverged from a common ancestor SIV_{sm}, use different gene products to suppress tetherin function: SIVmac uses Nef, whereas HIV-2 uses its envelope glycoprotein. Multiple presentations also discussed the mechanisms by which Vpu inhibits tetherin functions and the key amino acid motifs that mediate the Vpu–tetherin interaction. A functional "artificial tetherin" was also described; this hybrid molecule was generated by splicing different functional domains from various host molecules to reveal a highly potent synthetic that, expectedly, was completely resistant to HIV-1 Vpu.

Several interesting talks were given in the "RNA mechanisms" session this year. The interactions between viral RNA and Gag protein during HIV-1 particle assembly were captured using total internal reflection microscopy (TIRF) analyses by Dr. Paul Bieniasz' laboratory. This study revealed that viral RNA signals were first observed on cell surface, followed by Gag protein signals, suggesting that RNA binding is the nucleation event of Gag assembly. This work was presented by Dr. Nolween Jouvenet, who received the Andy Kaplan Award in 2009. Using confocal microscopy, the locations of viral RNA and Gag were studied in HIV-1- and FIV-based systems by Dr. Eric Poeschla's laboratory. Although RNA and Gag can be seen to colocalize near the plasma membrane in both HIV-1 and FIV, only HIV-1 Gag and RNA were colocalized near the perinuclear region. It is known that RNA secondary structures in the untranslated regions of the HIV-1 genome have essential roles in HIV-1 replication; the importance of RNA secondary structures in the coding region was explored by Dr. Ronald Swanstrom's laboratory. Mutants containing synonymous mutations in the HIV-1 genome predicted to alter secondary structures had decreased viral replication fitness compared with that of the wild-type genome, suggesting that these previously undefined secondary structures have important roles during viral replication.

R. Desrosiers

There were several surprising and intriguing studies that emerged during the meeting. A presentation by Dr. Massimo Pizzato indicated that the function of HIV-1 Nef can be replaced by an unrelated viral protein from murine leukemia virus (MLV), glycogag, perhaps as a consequence of convergent evolution. Another interesting study from Dr. Vinay Pathak's laboratory compared the connection subdomain of the reverse transcriptase between subtype B and CRF-01 AE HIV-1 and found that the AE connection domain can confer high-level AZT resistance in the presence of TAM (thymidine analog) mutations. This study reveals

P. Bieniasz, M. Malim

that the high divergence of the HIV-1 genome increases the complexity of the mechanisms of drug resistance; such studies are especially important because antiviral roll-out programs are beginning to be implemented in resource-poor countries that are mostly affected with nonsubtype B HIV-1 variants. Dr. Marc Johnson's laboratory reported that the expression of HIV-1 Vpu facilitated the incorporation and pseudotyping of gibbon ape leukemia virus (GALV) Env protein into HIV-1 particles; this is the first example of an accessory protein that may affect pseudotyping, potentially expanding the repertoire of pseudotyped viruses for use in basic cell biology as well as therapeutic gene therapy. Assembly near cell–cell contact sites has previously been reported in HIV-1. Dr. Walter Mothes' laboratory reported that MLV assembly is also facilitated near cell–cell contact sites; furthermore, the cytoplasmic tail of MLV Env promoted assembly near these sites. In a late-breaker insertion talk, Dr. Mark Yeager's laboratory presented results that purified TRIM5-α protein assembles into sheets in vitro that contained threefold symmetry. Interestingly, this sheet forms a complementary lattice with an in-vitro-assembled capsid lattice, providing insights into how TRIM5-α may interact with capsid to restrict HIV-1 replication.

Following its initial description last year that the karyopherin β-protein transportin-3 is an important HIV-1 cofactor, about half of the Uncoating/Nuclear Import session was dedicated to talks on the mechanism of transpotin-3 in lentiviral reverse transcriptase/preintegration complex nuclear translocation. The Integration session was composed of papers using a wide variety of techniques, including fluorescence imaging of nuclear preintegration complexes (Hope/Cereseto) as well as small-angle scattering studies of novel RSV (Anne Skalka) and HIV-1 (Bushman/van Duyne) structures. Dr. Peter Cherepanov's analysis of a new maedi-visna virus–LEDGFp75 cocrystal structure shed significant new light on the integration mechanism. Dr. Zeger Debyser's group presented two papers on the role of the critical LEDGFp75 integration cofactor, one that utilized DamID to determine a genome-wide map of LEDGFp75-binding sites and a second that, interestingly, redirected HIV-1 integration to novel sites via artificial proteins composed of the factor's integrase-binding domain fused to known chromatin-binding domains. This latter study demonstrates the ability to direct integration to predetermined sites, which might be useful down the road for targeted gene therapy.

Cocktails on Blackford lawn

The Retroviruses meeting is the premiere conference for retrovirologists who work on basic molecular and cellular biological aspects of infection. Several aspects of the meeting make it unique and essential for most retrovirologists to attend. It is the most scientifically intense meeting in the field, covering most of the topics of current retroviral research. Because the program is abstract-driven and emphasizes unpublished studies, only the most recent progress is discussed. Most of the presentations are given by postdoctoral fellows and graduate students; therefore, the meeting provides an excellent venue for trainees to present their work and to get feedback on their research. The format of the meeting also provides an opportunity for interactions between scientists during the question and answer part of oral presentations and during poster sessions. All of these features and the reputation of this meeting make it the premiere conference for basic research on retroviruses.

The meeting was funded in part by the National Institute of Allergy and Infectious Diseases, a branch of the National Institutes of Health.

PROGRAM

Entry/Envelope
Chairpersons: Y. Wu, *George Mason University, Manassas, Virginia;* R. Davey, *University of Texas Medical Branch, Galveston*

Uncoating/Nuclear Import
Chairpersons: A. Fassati, *University College London, United Kingdom;* N. Landau, *New York University School of Medicine, New York*

Apobec/RT
Chairpersons: J. DeStefano, *University of Maryland, College Park;* H. Matsuo, *University of Minnesota, Minneapolis*

Keynote Address: Use of SIV to Guide AIDS Vaccine Development Efforts
R. Desrosiers, *Harvard Medical School*

Integration
Chairpersons: Z. Debyser, *University of Leuven, Belgium;* H. Levin, *NICHD, National Institutes of Health, Bethesda, Maryland*

Trim/Accessory Proteins
Chairpersons: J. Luban, *University of Geneva, Switzerland;* O. Fackler, *Universitätsklinikum Heidelberg, Germany*

RNA Mechanisms
Chairpersons: L. Mansky, *University of Minnesota, Minneapolis;* R. Swanstrom, *University of North Carolina, Chapel Hill*

Pathogenesis
Chairpersons: R. Gummuluru, *Boston University School of Medicine, Massachusetts;* I. Singh, *University of Utah, Salt Lake City*

Keynote Address: Lessons from Four Decades of Research on Retrovirus Assembly
V. Vogt, *Cornell University*

Assembly
Chairpersons: F. Bouamr, *National Institutes of Health, Bethesda, Maryland;* H. Gottlinger, *University of Massachusetts Medical School, Worcester*

Tetherin/BST-2/CD317
Chairpersons: P. Cannon, *University of Southern California, Los Angeles;* D. Evans, *Harvard Medical School, Boston, Massachusetts*

Drug Resistance/Antivirals/Evolution
Chairpersons: V. Pathak, *NCI-Frederick, Maryland;* E. Freed, *NCI-Frederick, Maryland*

Workshop on Single-cell Techniques

July 16–19 55 Participants

ARRANGED BY **James Eberwine,** University of Pennsylvania Medical School
Xiaoliang Sunney Xie, Harvard University

X.S. Xie, J. Eberwine

The goal of this first workshop was to bring together scientists who work with single cells using different experimental paradigms and to discuss the progress that is being made. Nearly 60 scientists convened with 17 talks given. A panoply of cell types was discussed including bacteria, algae, plants, *C. elegans*, and mammalian cells. The range of techniques used to investigate single-cell biology that were discussed included single-cell dissection and transcriptomics, proteomics, lipidomics, and metabolomics, all at single-cell resolution. An important component of the meeting was a discussion of various imaging modalities including single-molecule resolution optics, PALM, STORM, and X-ray tomography. The importance of, and necessity for, microfabrication and nanotechnology was highlighted in several talks. With the large amount of data generated for any particular cell and the large number of cells that comprise a multicellular organism, the discipline of computational biology as an organizing and analysis component of single-cell biology was featured in nearly every presentation. The continued analysis of single-cell biology will undoubtedly lead to a better understanding of disease state, better production of biofuels, and insight into evolutionary mechanics that is unachievable using pooled cells.

This Workshop was sponsored in part by Carl Zeiss MicroImaging and the PENN Genome Frontiers Institute.

PROGRAM

Keynote Address: Connectomics in the Developing Nervous System
J. Sanes, *Harvard University, Cambridge, Massachusetts*

Single-cell Genomics and Transcriptomics
Chairperson: P. Blainey, *Stanford University, California*

Single-cell Proteomics and Metabolomics
Chairperson: J. Sweedler, *University of Illinois, Urbana*

Probing Single Live Cells
Chairperson: S. Frasier, *California Institute of Technology, Pasadena*

Probing Single Cells at the Nanometer Scale
Chairperson: J. Lippincott-Schwartz, *National Institutes of Health, Bethesda, Maryland*

Single-cell Modeling and Computation
Chairperson: A. Klein, *Harvard University Medical School, Boston, Massachusetts*

General Discussion

Group photo

Yeast Cell Biology

August 11–15 255 Participants

ARRANGED BY **Daniel Lew,** Duke University
Peter Pryciak, University of Massachusetts Medical School
Lois Weisman, University of Michigan

This conference was the 13th biannual international meeting devoted to diverse aspects of cell biology in yeasts. These model eukaryotes have served as a workhorse for investigation into widely conserved and fundamental features of eukaryotic cell biology. In addition to their well-known genetic and molecular tractability, this meeting highlighted how recent advances exploiting the impressive yeast knowledge base continue to expand the frontiers of cell biology. For example, an ingenious new strategy reported by Elmar Schiebel exploits the inherent folding rates of fluorescent proteins to monitor protein age in living cells. Another clever strategy reported by Dan Gottschling, dubbed the "mother enrichment program," allows a detailed look at how yeast cells themselves change with age, revealing an unexpectedly early onset of defects in a variety of cellular processes that are miraculously reversed by "rejuvenation" of daughter cells.

Several exciting presentations reported new findings resolving old mysteries, including how peroxisomes are generated (Tabak and Rachubinsky labs) and how secretory vesicles are ferried to their destinations (Weisman and Bretscher labs). Other talks reported surprising new twists in processes that were believed to be well understood. For example, presentations from M. Peter's lab revealed that a signaling scaffold protein can be switched, by a single phosphorylation event, to become a ubiquitin ligase and that pH has a hitherto unsuspected role in signaling glucose deprivation. A presentation from F. Cross reported unexpected "endocycles" of cell cycle regulators upon arrest of the cell cycle, and work from A. Gladfelter suggested that the same cell cycle controllers that coordinate nuclear and cytoplasmic events in yeast have novel roles promoting a lack of coordination between nuclei sharing the same cytoplasm in a multinucleate fungus.

Although traditionally focused on the Baker's yeast *Saccharomyces cerevisiae*, this year's meeting showcased the rapidly growing influence of the fission yeast *Schizosaccharomyces pombe*, particularly in the study of the cytoskeleton and cytokinesis. After more than 100 years of investigation, this exquisitely regulated process is finally yielding its secrets to an intense combination of experimental approaches ranging from biophysics to cell biology to genetics. Intriguingly, the force-producing mechanisms and the regulatory triggers for cytokinesis appear to differ between the two yeasts, and the advances reported at the meeting produced a set of new puzzles to solve for the next meeting.

G. Sprague, R. Miller

A. Ikui, A. Kitazono

The successes of the past two decades of research into diverse aspects of cell biology produced a knowledge base so large and detailed that it sometimes appeared as if different fields would become so specialized as to inhibit communication between investigators focusing on different areas. However, empowered by new systems biology approaches developed and road-tested in this most tractable of all eukaryotes, as well as the informed knowledge sharing promoted by the *Saccharomyces* genome database, there is now a trend in the opposite direction, toward a more holistic understanding of the organism. By bringing together investigators from all walks of cell biology who share common organism-specific approaches, this meeting has consistently helped to maintain open lines of communication and fostered our ability to integrate understanding and exchange insights from disparate areas. This was an intensely enjoyable meeting in which we learned a huge amount and delighted in the continuing new insights revealed by our beloved fungi, as well as the startling ingenuity and consistent innovation of our colleagues.

This meeting was funded in part by the National Science Foundation.

PROGRAM

Polarity and Cytokinesis
Chairpersons: A. Bretscher, *Cornell University, Ithaca, New York;* M. Peter, *Institute of Biochemistry, Zürich, Switzerland*

Cell Cycle and Proteolysis
Chairpersons: F. Cross, *The Rockefeller University, New York;* D. Morgan, *University of California, San Francisco*

Signaling Networks
Chairpersons: R. Brent, *The Molecular Sciences Institute, Berkeley, California;* M. Cyert, *Stanford University, California*

Membrane Trafficking
Chairpersons: S. Ferro-Novick, *HHMI/University of California, San Diego;* R. Hampton, *University of California, San Diego*

Chromosomes and Mitosis
Chairpersons: D. Koshland, *HHMI/Carnegie Institution of Washington, Baltimore, Maryland;* E. Schiebel, *University of Heidelberg, Germany*

Organelles
Chairpersons: B. Glick, *University of Chicago, Illinois;* J. Nunnari, *University of California, Davis*

Cytoskeleton
Chairpersons: D. Drubin, *University of California, Berkeley;* D. Pellman, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

Nuclear Organization
Chairpersons: J. Aitchison, *Institute for Systems Biology, Seattle, Washington;* D. Gottschling, *Fred Hutchison Cancer Research Center, Seattle, Washington*

S. Barva, S. Lee

M. Cyert, K. Whitworth, T. Aronin

Eukaroytic mRNA Processing

August 18–22 338 Participants

ARRANGED BY **Douglas Black,** HHMI/University of California, Los Angeles
Lynne Maquat, University of Rochester Medical Center
Joan Steitz, Yale University School of Medicine

D. Black, L. Maquat, J. Steitz

This seventh meeting presented and discussed recent developments in mRNA metabolism. As in the past, the regulation of alternative pre-mRNA splicing was a focus, as was the mechanism of spliceosome assembly and catalysis. Additional areas included mRNA 3′-end formation, mRNA decay, mRNA trafficking, and the coupling of RNA processing to transcription and export. Additional sessions covered genome-wide approaches to understanding RNA-mediated gene regulation and, for the first time, the control of microRNA (miRNA) biogenesis and mechanisms of RNA interference (RNAi).

In the splicing mechanisms session, several talks described new structural data on RNA recognition within the spliceosome or the overall conformation of the catalytic complex. Other results provided important new results on the role of metal ions in catalysis and the function of DEAH-box helicases in driving conformational changes during the catalytic cycle. The recognition of the branch point by the U2 small nuclear ribonucleoprotein (snRNP) and of exon sequences adjacent to the 5′ splice site was also described. Finally, a talk presented new examples of *trans*-splicing in *Drosophila* as identified by new deep-sequencing methods.

Several presentations on 3′-end formation focused on links between polyadenylation and transcription and quality control. Progress was described in characterizing the processing of the 3′ ends of histone mRNAs. Other talks examined the biogenesis and function of miRNAs. The regulation

Coffee break under canvas

of miRNAs by poly(ADP-ribose) and by cell-specific cofactors was described. Effects on miRNA processing in the nucleus by the spliceosome and heterogeneous nuclear RNP (hnRNP) proteins were presented, as were the roles of partner proteins in determining the specificity of dicer processing in the cytoplasm.

Talks in the RNA movement and RNA/protein interactions session examined a variety of important RNA-binding factors. Presentations described the different modes of recognition used to bind RNA. The coupling of export factor assembly to the disassembly of the 3′-processing complex and the coupling of poly(A)-binding and 3′-untranslated region (3′UTR)-binding factors to RNA export were discussed, as were mechanisms for the nuclear retention of RNA. The targeting and localization of mRNAs in the *Drosophila* embryo and to the endoplasmic reticulum (ER) and mitochondria and the role of splicing in these processes were also examined.

In splicing regulation, there has been significant progress in deciphering the detailed regulatory mechanisms, as well as the biological roles of particular factors and splice variants. Biochemical and yeast genetic approaches have identified specific points in the spliceosome assembly pathway affected by particular factors. Multiple talks presented new data on the autoregulation of splicing factors and the regulation of splicing during cellular differentiation. Another active area was the examination of roles for splicing regulation in human disease and the development of splicing targeted therapeutics.

In the RNA decay session, the nonsense-mediated decay pathway was a particular focus including work on its global regulation, its coupling to translation and to splicing via the exon junction complex, and the remodeling or recycling of NMD factors during decay. New studies of nuclear and viral pathways of mRNA decay were also presented covering both the known nuclear exosome pathway and a new pathway.

The major theme in the session on genome-wide approaches was regulatory networks. Newly refined array methods, new high-density sequencing methods, and the wider dissemination of methods for measuring genome-wide RNA binding by proteins were all evident. Combining these methods with RNAi approaches is allowing networks of processing events controlled by particular proteins or during particular developmental or physiological events to be assessed. Computational approaches to predicting regulation were also presented.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development branches of the National Institutes of Health, and by the National Science Foundation. The Laboratory would in addition like to thank the RNA Society for its support of this meeting.

PROGRAM

Splicing Mechanisms
Chairperson: B. Schwer, *Weill Cornell Medical College, New York*

3′ Ends and microRNAs
Chairperson: S. Buratowski, *Harvard Medical School, Boston, Massachusetts*

RNA Movement and RNA/Protein Interactions
Chairperson: A. Corbett, *Emory University School of Medicine, Atlanta, Georgia*

Splicing Regulation I
Chairperson: J. Valcárcel, *Centre for Genomic Regulation, Barcelona, Spain*

RNA Decay and Translation
Chairperson: H. Le Hir, *CNRS, Gif-sur-Yvette, France*

Coupled Processes
Chairperson: K. Neugebauer, *Max-Planck Institute for Cell Biology and Genetics, Dresden, Germany*

Splicing Regulation II
Chairperson: X.-D. Fu, *University of California, San Diego*

Genome-wide Approaches
Chairperson: B. Blencowe, *University of Toronto, Canada*

Mechanisms of Eukaryotic Transcription

August 25–29 407 Participants

ARRANGED BY **Stephen Buratowski,** Harvard Medical School
Barbara Graves, University of Utah
Steven Hahn, Fred Hutchinson Cancer Research Center

Regulation of gene transcription has a central role in the growth and development of eukaryotic organisms. Transcriptional responses occur as a consequence of cell signaling, environmental stresses, and developmental cues. The field of transcription encompasses a broad range of study from structural biology to developmental biology. This 10th meeting appropriately covered many aspects of eukaryotic transcription and brought together a diverse group of scientists who took part in eight plenary sessions and three poster sessions. The meeting began with a new session entitled Chromatin Dynamics that covered mechanisms of chromatin structure rearrangement that are essential for regulated gene expression. The next session, Coactivator Complexes and Activator Mechanisms, centered on the mechanisms of coactivator complexes that bridge transcription activators and the general transcription factors. The next two sessions discussed mechanisms of transcription, which revealed a new understanding of the transcription initiation process and the emerging importance of mechanisms used to regulate gene expression after transcription initiation. Histone Modifications and Transcription described new mechanisms whereby histone modifications lead to changes in transcription and chromatin organization. Regulation of Transcription Factor Activity and Specificity highlighted diverse mechanisms used to regulate transcription factor activity ranging from covalent modification to elaborate gene-regulatory networks controlling the expression of key transcription factors. The focus of the last two sessions turned to biological aspects of transcriptional regulatory mechanisms. Developmental Regulation described transcriptional regulatory mechanisms that control development, and Signaling Mechanisms described novel signaling pathways that regulate transcription and oncogenesis. The meeting was very well received by the participants and will occur again in 2011.

The meeting was funded in part by grants from the National Institutes of Health and the National Science Foundation.

PROGRAM

Chromatin Dynamics
Chairperson: J. Workman, *Stowers Institute for Medical Research, Kansas City, Missouri*

Coactivator Complexes and Activator Mechanisms
Chairperson: C. Wolberger, *HHMI/Johns Hopkins University School of Medicine, Baltimore, Maryland*

Initiation, Elongation, and Termination I
Chairperson: I. Grummt, *German Cancer Research Center, Heidelberg*

Initiation, Elongation, and Termination II
Chairperson: D. Brow, *University of Wisconsin, Madison*

Histone Modifications and Transcription
Chairperson: J. Conaway, *Stowers Institute for Medical Research, Kansas City, Missouri*

Regulation of Transcription Factor Activity and Specificity
Chairperson: K. Struhl, *Harvard Medical School, Boston, Massachusetts*

Developmental Regulation
Chairperson: K. Jones, *Salk Institute for Biological Studies, La Jolla, California*

Signaling Mechanisms
Chairperson: K. Zaret, *University of Pennsylvania School of Medicine, Philadelphia*

Eukaryotic DNA Replication

September 1–5 338 Participants

ARRANGED BY **Stephen P. Bell,** HHMI/Massachusetts Institute of Technology
Joachim Li, University of California, San Francisco

J. Li, S. Bell

This 11th biannual meeting was the second meeting that expanded the scope of the meeting by including presentations on DNA repair and checkpoint processes that monitor the integrity of the DNA replication process. The meeting demonstrated the remarkable progress that has been made in the past 2 years. Accordingly, this meeting is crucial in bringing together an international array of researchers investigating all aspects of eukaryotic DNA replication and genome maintenance. The most recent advances in the field were presented together with new approaches for analyzing DNA replication, making this meeting the most important in the field. A total of 338 investigators participated in the 10 scientific sessions, with a total of 275 platform and poster presentations. The eight platform and two poster sessions were marked by spirited and enthusiastic exchanges of new results. In the scientific sessions, many audience members participated in the question and answer sessions, and the poster sessions were both of very high quality and well attended.

Sessions held at the meeting are listed below. In particular, the meeting illustrated the incorporation of structural approaches to understand the events of initiation, the reconstitution of many of the key steps in the replication initiation and DNA-repair events, and the incorporation of genome-wide methodology to understand the complexities of metazoan DNA replication and repair. In addition, the presentations illustrated our increasing understanding of the control of DNA replication during development as well as the close coordination between the DNA repair and checkpoint processes and DNA replication initiation and elongation.

Essential funding for the meeting was provided by the National Cancer Institute, a branch of the National Institutes of Health, and the National Science Foundation.

PROGRAM

Origin Activation and Replisome Assembly
Chairperson: H. Araki, *National Institute of Genetics, Mishima, Japan*

Chromatin and Origin Timing
Chairperson: J. Walter, *Harvard Medical School, Boston, Massachusetts*

Cell Cycle Controls and Genomic Instability
Chairperson: T. Orr-Weaver, *Whitehead Institute, Massachusetts Institute of Technology, Cambridge*

Replisome Structure and Function
Chairperson: S. Gasser, *Friedrich Miescher Institute, Basel, Switzerland*

Postreplicative Repair and Lesion Bypass
Chairperson: T. Kunkel, *National Institutes of Health/NIEHS, Research Triangle Park, North Carolina*

Origin Selection and Pre-RC Assembly
Chairpersons: A. Dutta, *University of Virginia, Charlottesville;* J. Hamlin, *University of Virginia, Charlottesville*

Chromatin and Development
Chairperson: M. Mechali, *Institute of Human Genetics, CNRS, Montpellier, France*

Response to Replication Stress
Chairpersons: P. Burgers, *Washington University School of Medicine, St. Louis, Missouri;* A.-K. Bielinsky, *University of Minnesota, Minneapolis*

Microbial Pathogenesis and Host Response

September 8–12 247 Participants

ARRANGED BY **Andrew Camilli,** Tufts University School of Medicine
Brendan Cormack, John Hopkins University School of Medicine
Theresa Koehler, University of Texas–Houston Medical School

B. Cormack, T. Koehler, A. Camilli

Despite advances in modern health care, infectious diseases continue to be major causes of human morbidity and mortality. The evolution of microbial pathogens with humans has resulted in complex interactions that impact the struggle between the infectious invader and the susceptible host. Increased understanding of these interactions with the goal of developing new therapeutics and preventive strategies requires collaborative and interdisciplinary scientific approaches. This meeting brought together a diverse group of international scientists who approach the study of bacterial and fungal pathogens from a broad range of perspectives. Investigators from the disciplines of molecular microbiology, eukaryotic cell biology, immunology, and genomics, and representing academia, industry, and the public health sector, shared recent findings concerning microbial and host aspects of infectious diseases.

The meeting focused on bacterial and fungal pathogens and the host response and defense to these invading microbes. Oral sessions were topic-based and included studies of multiple and diverse organisms. Areas covered included structural analysis of virulence factors, host immune response and immune evasion strategies, microbial communities, and reservoirs, microbial metabolism and physiology and microbial genomics. Speakers for each session were a mixture of established leaders in the field and young investigators; 40% of the speakers, including postdoctoral fellows and graduate students, were chosen from submitted abstracts. Dr. Olaf Schneewind, Professor and Chair of the Department of Microbiology at the University of Chicago, presented the keynote address. Dr. Schneewind is an internationally recognized leader in microbial pathogenesis. His presentation addressed processing of microbial cell-surface proteins and attachment to peptidoglycan by the sortase enzyme, as well as immune recognition of these proteins and their application to vaccine development for gram-positive organisms.

Poster session in the Grace lobby

The informal atmosphere combined with the broad perspectives of the meeting participants resulted in a free flow of novel and refreshing ideas on pathogenesis, with the atmosphere of a small meeting. Active questioning and discussion followed all oral presentations, was evident throughout the posters sessions, and continued during a wine and cheese reception and other social gatherings. We strongly encouraged submission of abstracts by junior researchers in the field and many young investigators were in attendance. Some of these interactions have already produced fruitful scientific collaborations.

This meeting was partially supported by funds from the National Institute of Allergy and Infectious Diseases.

S. Chen, A. Sikora

PROGRAM

Structural Insights into Virulence
Chairperson: P. Ghosh, *University of California, San Diego*

Metabolism and Virulence
Chairperson: J. Slauch, *University of Illinois, Urbana, Champaign*

Immune Evasion
Chairperson: J. Cox, *University of California, San Francisco*

Reservoirs and Transmission
Chairperson: C. Kumamoto, *Tufts University, Boston, Masaschusetts*

The Immune Host
Chairperson: M. Starnbach, *Harvard Medical School, Boston, Massachusetts*

Communities
Chairperson: P. Watnick, *Children's Hospital, Harvard Medical School, Boston, Massachusetts*

Keynote Address: Cell-surface Proteins at the Microbial–Host Interface
O. Schneewind, *University of Chicago, Illinois*

Genomes
Chairperson: C. Fraser-Liggett, *University of Maryland School of Medicine, Baltimore*

A. Pezzicoli

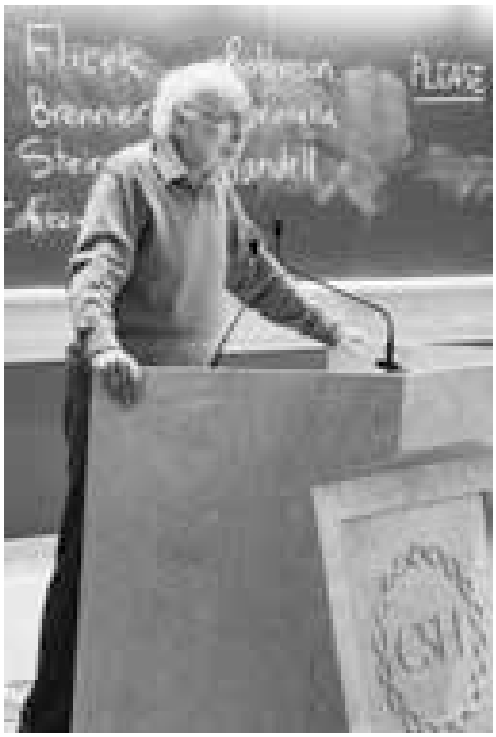
R. Miller-May, J. O'Loughlin

Personal Genomes

September 14–17 200 Participants

ARRANGED BY **George Church,** Harvard University
Paul Flicek, European Bioinformatics Institute
Richard Gibbs, Baylor College of Medicine
Elaine Mardis, Washington University School of Medicine

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 including (1) technical status of sequencing whole genomes: successes, problems, new developments, new approaches on the way; (2) making sense of the content of whole genomes: what are we learning about human genetics from genome scale studies?; (3) applications of whole-genome studies: How are genome-scale studies providing new insights on clinical genetics?; and (4) preparing for the "whole-genome" world: What happens when everyone has their genome sequenced? The meeting featured a combination of talks and posters and included 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 considerable enthusiasm of the audience and the appearance of two major editorials citing details of the conference in subsequent issues of *Nature* magazine. The collective decision was taken to hold the meeting for a third time at Cold Spring Harbor in the fall of 2010.



J.D. Watson

This meeting was funded in part by Roche-454 Sequencing and Illumina, Inc.

PROGRAM

Keynote Address: Setting the Tone for Personal Genomes
G. Church, *Harvard University, Cambridge, Massachusetts*

Introductory Remarks
J.D. Watson, *Cold Spring Harbor Laboratory*
G. Church, *Harvard University:* Overview of technologies and status/application of personal genome sequencing.
T. Hudson, *Ontario Institute for Cancer Research:* Genetic analysis of 20 loci implicated in colorectal cancer.

Whole-genome Studies and Cancer
Chairpersons: E. Mardis, *Washington University School of Medicine, St. Louis, Missouri;* L. Chin, *Harvard Medical School, Boston, Massachusetts*


E. Lai, C.T. Caskey

Genomes in Inherited Disease
Chairpersons: R. Gibbs, *Baylor College of Medicine, Houston, Texas;* R. Lifton, *Yale University School of Medicine, New Haven, Connecticut*

Keynote Address: Inflection Point for Genome Science
C.T. Caskey, *University of Texas Health Science Center*

TPANEL: New Ethical Challenges and Personal Genomes
M. Angrist, *Duke University, Durham, North Carolina:* Historical overview of personal genomes.
J. Lupski, *Baylor College of Medicine, Houston, Texas:* My personal genome experience.
V. Hughes, *New York, New York:* Potential impact of personal genomics in a disease focus group.
A. McGuire (co-chair), *Baylor College of Medicine, Houston, Texas:* Ethical issues related to clinical integration and research use of personal genomes.
P. Reilly (co-chair), *Third Rock Ventures, Massachusetts:* Ethical issues moving forward.

Scanning the Exome and Personal Transcriptomes
Chairpersons: J. Shendure, *University of Washington, Seattle;* C. Nusbaum, *Broad Institute of MIT and Harvard, Cambridge, Massachusetts*

Data Analysis and Visualization Tools
Chairpersons: P. Flicek, *European Bioinformatics Institute, Hinxton, United Kingdom;* S. Brenner, *University of California, Berkeley*

Future Technologies for Personal Genomics
Chairpersons: R. Wilson, *Washington University, St. Louis, Missouri;* S. Quake, *Stanford University, California*

P. Reilly, M. Pollock

T. Hudson, A. Metspalu

V. Hughes

A. McGuire

J. Lupski, L. Richards

Stem Cell Biology

September 22–26 242 Participants

ARRANGED BY **Konrad Hochedlinger,** Massachusetts General Hospital
Brigid Hogan, Duke University Medical Center
Allan Spradling, HHMI/Carnegie Institution

This inaugural meeting was designed to bring together researchers taking a broad range of approaches to analyzing stem cells from a diverse collection of tissues and experimental organisms, both in vivo and in vitro. Regardless of their experimental strategy, speakers agreed that the field's paramount goal is to understand stem cell behavior in vivo during tissue development, homeostasis, and repair, including cellular reprogramming. The key subject areas of the meeting are listed below. A prominent goal of the meeting was to juxtapose insights from invertebrate model systems boasting favorable biology and powerful experimental tools with those from mammalian and human systems with a long history of study and great clinical relevance. A significant fraction of the material presented was unpublished and some fascinating discoveries were described for the first time. The meeting highlight was an exceptional keynote presentation containing a large amount of new data of central relevance to stem cell biology. The ensuing discussion had to be cut off after 20 minutes to make way for the concert. Overall, meeting participants were extremely positive, a typical comment being: "This is the most biologically focused stem cell meeting I have ever attended."

The meeting included participants from at least 20 different countries representing Australia, North America, South America, Asia, South Asia, and Europe. Although many attendees are renowned in their fields, a large number of participants were young scientists. In addition to the high-quality program, the meeting attracted these young participants because nearly half the speakers, including many junior researchers, were selected from the submitted abstracts. A highly interactive atmosphere was successfully created by limiting all talks to 20 minutes, leaving one-third of the alloted time for discussion, which often expanded greatly on the formal presentations.

A. Spradling, B. Hogan, K. Hochedlinger

J. Rossant, S. Morrison

PROGRAM

Anatomy and Function of the Stem Cell Niche I
Chairperson: K. Hochedlinger, *Massachusetts General Hospital, Boston*

Anatomy and Function of the Stem Cell Niche II
Chairperson: D.L. Jones, *Salk Institute for Biological Studies, La Jolla, California*

Epithelial Stem Cells I
Chairperson: B. Hogan, *Duke University Medical Center, Durham, North Carolina*

Epithelial Stem Cells II
Chairperson: J. Rossant, *The Hospital for Sick Children, Toronto, Canada*

Cell Lineage Determination, Differentiation, and Plasticity
Chairperson: L. Zon, *Children's Hospital, Boston, Massachusetts*

Tissue Regeneration, Repair, and Reprogramming
Chairperson: M. Busslinger, *Research Institute of Molecular Pathology, Vienna, Austria*

Tissue Regeneration, Maintenance, and Repair
Chairperson: Y. Yamashita, *University of Michigan, Ann Arbor*

Stem Cell Self-renewal and Differentiation
Chairperson: H. Clevers, *Hubrecht Institute, Utrecht, The Netherlands*

Keynote Address: Lgr5 Intestinal Stem Cells in Self-renewal and Cancer
H. Clevers, *Hubrecht Institute*

Pluripotency
Chairperson: A. Spradling, *HHMI/Carnegie Institution, Baltimore, Maryland*

C. Blanpain, V. Greco

S. Ellis, J. Kung

Neurobiology of *Drosophila*

September 29–October 3 441 Participants

ARRANGED BY **Vivian Budnik,** University of Massachusetts
Paul Taghert, Washington University Medical School

P. Taghert, V. Budnik

As in previous years, the goal of this meeting was to foster communication of ideas, techniques, and new discoveries within the field of *Drosophila* neurobiology. The meeting was structured with platform and poster presentations by a variety of researchers including graduate students, postdoctoral fellows, and junior and senior faculty. The topics for the platform sessions were chosen to reflect the areas of *Drosophila* neurobiology in which cutting-edge advances are being made: neuronal cell development, neural circuits and function, simple and complex behaviors, sensory systems, synaptic transmission, and the evolution of neural systems. A small number of abstracts submitted in each of these areas were selected by the respective session chairs for platform presentations, and the rest were presented as posters. The research reported used a wide range of techniques, including genetic, molecular, cellular, biochemical, physiological, and behavioral approaches to basic questions of nervous system development and function. Among the highlights of the meeting were the creative ways in which researchers are using *Drosophila* to understand the molecular and cellular underpinnings of many different physiological and pathological processes. A focal point in the meeting was the Keynote Address by Gerry Rubin who described the substantial efforts to advance *Drosophila* neurobiology at Janelia Farm. In addition, the meeting included reports of important advances in the development of the nervous system, the perception of external stimuli, in particular olfactory cues, by the fly, and the design of new technology. The Elkins plenary lecture was presented by Young Kwon from Craig Montell's laboratory. He spoke about the physiology and genetics of temperature-sensitive channels. The environment of the meeting allowed many opportunities for informal discussions among all participants. The high quality of the presentations, the development of novel techniques, and the exciting new directions of *Drosophila* research demonstrate the vitality of this area. Discussions at the meeting led to the sharing of ideas that were valuable to everyone in the field.

A. Stewart, M. Rolls

The meeting was funded in part by the National Institute of Neurological Disorders and Stroke and the Eunice Kennedy Shriver National Institute for Child Health and Human Development, branches of the National Institutes of Health.

PROGRAM

Development and Metamorphosis of the Brain
Chairperson: J. Kumar, *Indiana University, Bloomington*

Behavior (Reproductive, Locomotor, Appetitive, Social, Aggressive)
Chairperson: J. Levine, *University of Toronto, Canada*

Elkins Memorial Lecture: TRP Channels Required for Sensing Temperature and Insect Repellents
Y. Kwon, *Johns Hopkins School of Medicine*

Neural Circuits: Assembly and Function
Chairperson: I. Meinertzhagen, *Dalhousie University, Halifax, Canada*

Sensory Systems: Coding and Topographic Mapping
Chairperson: S. Tsunoda, *Colorado State University, Fort Collins*

Genome Studies and Neural Evolution
Chairperson: S. Reppert, *University of Massachusetts Medical School, Worcester*

Keynote Address: A Brief Overview of Ongoing Work on the Fly Brain at Janelia Farm
G. Rubin, *HHMI/Janelia Farm Research Campus*

Synapse Development and Plasticity
Chairperson: J. Sierralta, *Universidad de Chile, Santiago*

Higher Brain Functions (Learning, Memory, Sleep, Attention, Choice)
Chairperson: K. Han, *Pennsylvania State University, University Park*

H. Lawal, S. Aust, P. Halder, A. Ziegler

A. Wise, T. Venkates

J. Goto, K. Kitamoto

J. Levine, G. Rubin

Cell Death

October 6–10 267 Participants

ARRANGED BY **J. Marie Hardwick,** Johns Hopkins University
Shigekazu Nagata, Graduate School of Medicine, Kyoto University
Junying Yuan, Harvard Medical School

J. Yuan, J.M. Hardwick

The meeting opened with two Keynote presentations delivered by Junying Yuan and Doug Wallace, an expert on the molecular basis of mitochondrial disease. Junying Yuan provided an overview and articulated the frontiers of the field before describing her recent work on a new programmed necrotic cell death pathway, its key molecular players, and small molecule inhibitors, which has opened new therapeutic opportunities previously thought to be impossible based on the assumption that necrosis is a nonprogrammed and uncontrollable cell death. In addition to two poster sessions, there were eight oral sessions covering the topics of developmental cell death (organismal and immunological), apoptotic and nonapoptotic cell death pathways (autophagy, necrosis, inflammatory), new therapeutic targets (including new evidence for death receptors and caspases in Alzheimer's disease, presented in the oral and poster sessions), regulation of metabolism and mitochondrial dynamics by cell death factors, noncanonical functions of cell death regulators in the nervous system, systems biology approaches to understanding cell death, and a range of therapeutic strategies to induce cell death in cancer cells, backed by both basic science and clinical evidence.

Speakers from around the globe included high-profile investigators who were both invited speakers/chairs and abstract presenters. These included Craig Thompson, Beth Levine, Vishva Dixit, Marc Tessier-Lavign (CSO, Genentech), Richard Youle, Doug Green, Yigong Shi, Scott Lowe, Hermann Steller, Jerry Adams, Eileen White, and many others who spoke on a range of topics and model systems as they relate to mechanisms of cell death. Despite the many exciting new discoveries presented here, it was also clear that many of the major questions in this field remain unanswered, including those concerning the central molecular players in apoptosis.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.

M. Bots, J. Zuber, L. Dow

H. Steller, H. Engleberg-Kulka

PROGRAM

Keynote Addresses
Chairperson: J.M. Hardwick, *Johns Hopkins University, Baltimore, Maryland*
J. Yuan, *Harvard Medical School, Boston, Massachusetts:* Mechanisms of programmed cell death: From apoptosis to necroptosis.
D.C. Wallace, *University of California, Irvine:* Mitochondria, bioenergenetics, and epigenoemics in health and disease.

Developmental Cell Death: Organismal and Immunological
Chairperson: S. Nagata, *Kyoto University Graduate School of Medicine, Japan*

Autophagy, Necrosis, and Inflammation
Chairpersons: E. White, *Rutgers University, New Brunswick, New Jersey;* B. Levine, *University of Texas Southwestern Medical Center, Dallas*

Posttranslational Regulation of Cell Death and Therapeutic Targets
Chairpersons: V. Dixit, *Genentech Inc., South San Francisco, California;* S. Kornbluth, *Duke University School of Medicine, Durham, North Carolina*

Metabolism at the Interface of Death and Survival
Chairpersons: C. Thompson, *University of Pennsylvania, Philadelphia;* J. Lieberman, *Harvard Medical School, Boston, Massachusetts*

Structure, Biochemistry, and Genetics of Cell Death
Chairpersons: Y. Shi, *Tsinghua University, Beijing, China;* D. Andrews, *McMaster University, Hamilton, Canada*

Cell Death Signaling Pathways
Chairpersons: D. Green, *St. Jude Children's Research Hospital, Memphis, Tennessee;* P. Sorger, *Havard Medical School, Boston, Massachusetts*

Mitochondria: Mediators of Death and Survival
Chairpersons: R. Youle, *National Institutes of Health/NINDS, Bethesda, Maryland;* N. Daniel, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Noncanonical Functions of Cell Death Factors: Neurons to Plants
Chairpersons: M. Tessier-Lavigne, *Genentech Inc., South San Francisco, California;* E. Jonas, *Yale University, New Haven, Connecticut*

Cancer Cell Death Mechanisms: Bench to Bedside
Chairpersons: S. Lowe, *Cold Spring Harbor Laboratory;* Z. Fuks, *Memorial Sloan-Kettering Cancer Center, New York*

E. Jonas, T. Rostovtseva

L. Grantham

Genome Informatics

October 27–30 329 Participants

ARRANGED BY **Michele Clamp,** Broad Institute of MIT and Harvard
James Kent, University of California, Santa Cruz

M. Clamp, J. Kent

The ninth Cold Spring Harbor Laboratory/Wellcome Trust conference continues to highlight the latest developments in genome research and, once again, was a vital and exciting meeting. This conference series began at the Wellcome Trust Conference Center in Hinxton, United Kingdom in 2001 and now alternates between the United Kingdom and the United States. The series follows a format similar to traditional Cold Spring Harbor meetings, in that the majority of oral presentations are drawn from openly submitted abstracts.

The explosion of biological data requires a concomitant increase in the scale and sophistication of information technology. This ranges from the storage of data and associated data models, to the design of effective algorithms to uncover nonobvious aspects of these data sets, to ontologies to concisely describe biological information, and finally to software systems to support curation, visualization, and exploration. The conference brought together some of the leading scientists in this growing field, and researchers from other large-scale information handling disciplines were also invited to attend. The key topics of the meeting are listed below. For the first time, we had a session on microbial pathogens, a field with which most bioinformaticians are unfamiliar, and it generated much excitement and discussion. The keynote speaker was Professor Lincoln Stein, who addressed understanding genome-scale data sets with reactome. In all, more than 325 participants attended, with more than 30% of delegates coming from outside North America, a highly unusual statistic for a U.S. meeting, and the meeting hosted a record 215 scientific presentations in talks and posters.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health.

PROGRAM

Assembly and Informatics for New Sequencing Technologies
Chairpersons: I. Birol, *BC Cancer Agency, Vancouver, Canada;* M. Brudno, *University of Toronto, Canada*

Epigenomics and Gene Regulation
Chairpersons: E. Segal, *Weizmann Institute of Science, Rehovot, Israel;* X.S. Liu, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Data Management, Text Mining, Curation, and Visualization
Chairpersons: G. Micklem, *University of Cambridge, United Kingdom;* D. Church, *National Center for Biotechnology Information, Bethesda, Maryland*

Comparative and Evolutionary Genomics
Chairpersons: A. Regev, *Broad Institute of MIT and Harvard, Cambridge, Massachusetts;* C. Ponting, *Oxford University, United Kingdom*

Keynote Address: Understanding Genome-scale Data Sets with Reactome
L. Stein, *Ontario Institute for Cancer Research*

Population and Statistical Genomics
Chairperson: R. Nielsen, *University of California, Berkeley*

Transcriptomics and Medical Genomics
Chairperson: J. Parkhill, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Harnessing Immunity to Prevent and Treat Disease

November 11–14 133 Participants

ARRANGED BY **Kathrin Jansen,** Wyeth Pharmaceuticals
Robert Seder, Vaccine Research Center, NIH/NIAID
Susan Swain, Trudeau Institute

R. Seder, J. Banchereau

This fifth winter conference, previously called Molecular and Immunological Approaches to Vaccine Design, focused on new findings about the basic regulation of innate and adaptive immunity in the context of infectious and autoimmune disease and cancer and how the immune system might be harnessed to tackle each of these. Indeed, the first keynote speaker, Jim Allison, showed promising results on clinical trials using strategies to block CTLA-4, which unleashed T cells' improved response to certain cancers and restricted their growth. He also highlighted the role of several additional co-stimulatory pathways that could influence T-cell immunity.

The first session on innate immunity covered our increasing appreciation of the many roles of innate cells, including natural killer (NK) cells and dendritic cells (DC) of many subsets. It emerged that many of the regulatory mechanisms determining innate cell functions, such as cytokines interleukin-10 (IL-10), IL-12, type I interferon (IFN), and IL-21, are those known to regulate both B and T lymphocytes. Several speakers throughout this session highlighted the importance that efficient activation of antigen-presenting cells, such as specific DC subsets, had for influencing an effective response in which T cells, secreting multiple effector cytokines such as IFN-β, TNF-α (tumor necrosis factor-α), and IL-2, are generated. Furthermore, new data were presented showing that memory T cells can induce innate inflammation, independent of pathogen recognition pathways.

These topics were further explored in the second session on vaccines and adjuvants for noninfectious diseases. Data were presented showing that DNA vaccination may be a promising approach for treatment of autoimmune disease such as diabetes or multiple sclerosis through undefined mechanisms. In addition, it may be possible to harness adaptive immune responses to block development of Alzheimer's. Although treatments for all these disease are still some time away, this is an increasingly exciting possibility.

There were two sessions on T-cell immunity which revealed that the breadth of CD4 and CD8 T-cell responses are much greater than previously considered. The ability of the regulatory T cell to modulate immune responses becomes more and more apparent, especially in mucosal sites where they have a critical role. Roles for Th17 in repair have become clearer. The pathways of differentiation of CD4 (which often apply to CD8 as well) are also revealed to be at multiple levels. For instance, it seems that the T cells that enter B-cell follicles (T_{FH}) only turn into cells helping germinal center formation by turning on a program involving down-regulation of one factor, BLIMP, and up-regulation of an opposing factor, BCL-6. This decision seems to be location dependent. In addition, T:B cognate interaction seems to be necessary for the final differentiation to mature T_{FH}, which supports germinal center formation and the production of high-affinity isotype-switched antibodies that have key roles in protective immunity. Another program involving BLIMP and opposed by T-BET (a member of the T-box family) seems to control exhaustion of T cells. Pathways to memory T-cell formation are also being investigated and the mTOR pathway seems to have a key role.

As we better understand the kinds of immune responses and the regulatory pathways determining both effector and memory generation of different types, we should be able to translate this knowledge into more effective vaccine strategies tailored to particular pathogens and targets such as tumors. By bringing together the vaccine designers and those discovering basic mechanisms, we hope to inform and energize both groups so that translation can occur as rapidly as possible. The enthusiastic participation of attendees in discussions, in and outside the formal conference sessions, suggests that this goal was achieved.

This meeting was funded in part by the National Institute of Allergy and Infectious Diseases, a branch of the National Institutes of Health, and Wyeth Research Institute.

PROGRAM

Keynote Addresses: Perspectives on Immunotherapy for Cancer
R. Steinman, *The Rockefeller University*
J. Allison, *Memorial Sloan-Kettering Cancer Center*

Innate Immunity and Adjuvants
Chairperson: B. Pulendran, *Emory University, Atlanta, Georgia*

Keynote Address: Challenges to Vaccination
P. Offit, *Children's Hospital of Philadelphia*

Vaccines and Adjuvants for Noninfectious Diseases
Chairperson: D. Selkoe, *Harvard Medical School, Boston, Massachusetts*

Immunity to Infectious Disease I
Chairperson: J. Kolls, *Louisiana State University Health Sciences Center, New Orleans*

Vaccines for Challenging Diseases and New Threats
Chairperson: K. Subbarao, *National Institutes of Health/NIAID, Bethesda, Maryland*

Keynote Address: Development of a Conjugated Polysaccharide Vaccine that Affords Expanded Worldwide Coverage Against Pneumococcal Disease
E. Emini, *Wyeth Pharmaceuticals*

Keynote Address: CD8 T-cell Immunity to Virus
R. Ahmed, *Emory University School of Medicine*

Immunity to Infectious Disease II
Chairperson: R. Seder, *National Institutes of Health/NIAID, Bethesda, Maryland*

M. Eichelberger, A. Hassantougighi

K. Jansen, D. Selkoe

L. Richert

In Vivo Barriers to Gene Delivery

November 18–21 76 Participants

ARRANGED BY **Nori Kasahara,** UCLA Geffen School of Medicine
Stephen Russell, Mayo Clinic College of Medicine
James M. Wilson, University of Pennsylvania

G. Kueberuwa, S. Russell

It is now 20 years since the first FDA-approved human gene transfer study, but this extremely promising field of endeavor is still nowhere close to realizing its full therapeutic potential. There have been a few notable successes, such as the use of autologous hematopoietic stem cells transduced with the interleukin-2 (IL-2) γ-chain or adenosine deaminase for the treatment of severe combined immunodeficiency. However, the dream of genetically based therapies for the treatment of a multitude of other more common human ailments has not yet been achieved. Although clinical "home runs" have been few and far between, the scientific understanding and the technological underpinning of gene therapy have been moving forward in leaps and bounds, and there is currently a great deal of optimism that we are entering an era in which we will see increasing numbers of clinically approved gene therapy products. This conference provided a forum for the presentation of the most recent advances in technologies and approaches used for monitoring vector trafficking, evading neutralizing antibodies, avoiding vector mislocalization, increasing extravasation, enhancing persistence, improving the manufacturing yield, or otherwise addressing the barriers that interfere with the accuracy and efficiency of gene delivery and the durability of expression.

This unique forum was designed to stimulate future research studies in this important area and to promote interactions among researchers in diverse basic scientific areas relevant to this field. The conference was held the week before Thanksgiving and attracted 76 participants, including many eminent field leaders and a strong showing of more junior scientists (more than 50% of attendees were postdoctoral scientists or Ph.D. students). Overall, the meeting was considered a great success by those who attended. This was a highly interactive group of scientists that engaged in stimulating discussions after each of the oral presentations and very lively interactions at the poster sessions and, of course, in the bar after each of the evening sessions. Throughout the meeting, it was clear that the field of gene therapy has reached a very exciting stage in its development, and we expect to see significant progress addressing the in vivo barriers to gene delivery in the years ahead.

PROGRAM

Keynote Address: AAV Immunity: Antibodies and Inflammation
J. Wilson, *University of Pennsylvania*

Monitoring Gene Delivery and Expression
Chairpersons: S. Carlson, *Mayo Clinic, Rochester, Minnesota;* R. Blasberg, *Memorial Sloan-Kettering Cancer Center, New York*

Mislocalization, Sequestration, and Targeting
Chairpersons: A. Baker, *University of Glasgow, United Kingdom;* A. Srivastava, *University of Florida, Gainesville*

Persistence
Chairpersons: J. Bartlett, *Research Institute at Nationwide Children's Hospital, Columbus, Ohio;* M. Sadelain, *Memorial Sloan-Kettering Cancer Center, New York*

Extravasation and Tissue Penetration
Chairpersons: N. Kasahara, *University of California Geffen School of Medicine, Los Angeles;* L. Seymour, *University of Oxford, United Kingdom*

Bench to Bedside
Chairpersons: M. Serabian, *Food and Drug Administration, Rockville, Maryland;* K. High, *Children's Hospital of Philadelphia, Pennsylvania*

Keynote Address
L. Naldini, *San Raffaele Telethon Institute for Gene Therapy*

Vector Neutralization
Chairpersons: M. Agbandje-McKenna, *University of Florida, Gainesville;* M. Perreau, *Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland*

D. McCarty

D. Dingli, H. Li

Rat Genomics and Models

December 2–5 142 Participants

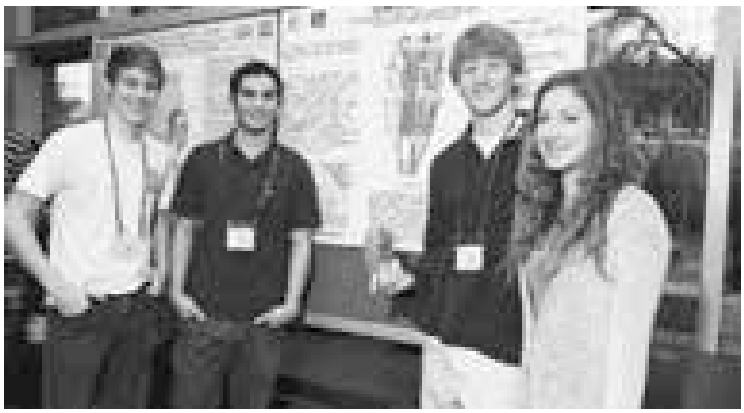
ARRANGED BY **Edwin Cuppen,** Hubrecht Institute
Norbert Hübner, Max-Delbrück Center for Molecular Medicine
Anne Kwitek, University of Iowa
James Shull, University of Wisconsin

J. Shull, N. Hübner, A. Kwitek, E. Cuppen

This sixth winter biotechnology conference has been held biannually since 1999 at the Cold Spring Harbor Laboratory. This conference series is the first in the United States to focus exclusively on the unique physiological relevance of the rat as a model organism for biomedical research into the molecular origins of human diseases. A complementary meeting outside of the United States is held in the alternating years, with the most recent meetings being held in Hinxton, United Kingdom (2008), Melbourne, Australia (2006), and Kyoto, Japan (2004). The primary goals of this meeting were (1) to promote interactions between biomedical researchers who utilize rat models in the study of physiology, pathophysiology, toxicology, immunology, neuroscience, and oncology and (2) to provide an interface between the research community and the various public and commercial entities that support biomedical research in which rat models are utilized.

The meeting was organized into oral presentations by invited speakers, speakers selected following evaluation of submitted abstracts, poster sessions, and workshops. Keynote lectures were presented by Dr. Michael Gould, Svante Pääbo, and Matthias Mann. The remaining oral presentations were organized into six sessions based on diseases or technologies. The key topics and their chairs are presented below.

Dr. Hübner led a workshop devoted to genome databases and data mining tools. In this workshop, Dr. Elizabeth Worthey presented an overview of the rat genome viewer developed by the Rat Genome Database, and Dr. Kim Pruitt from the National Center for Biotechnology Information presented a summary of new data mining tools developed within that organization. Finally, 57 abstracts were presented in an interactive poster session.



Dolan DNA Learning Center poster presenters

It is clear that this conference serves an important role in meeting the needs of those in the biomedical research community who use rat models in genetics and genomics-based research. The organizers and attendees expressed high enthusiasm for continuing the present biannual meeting format at the Cold Spring Harbor Laboratory and other sites in the future.

This meeting was funded in part by the National Heart, Lung, and Blood Institute, a branch of the National Institutes of Health.

PROGRAM

Tumor Biology
Chairpersons: J. Shull, *University of Wisconsin, Madison;* C. Walker, *University of Texas M.D. Anderson Cancer Center, Smithville*

Keynote Address: Susceptibility to Breast Cancer: A Complex Trait
M. Gould, *University of Wisconsin, Madison*

Metabolism/Inflammation
Chairpersons: Å. Lernmark, *Lund University, Sweden;* T. Aitman, *Imperial College, London, United Kingdom*

Workshop: Databases
Chairperson: N. Hübner, *Max-Delbrück Center for Molecular Medicine, Berlin, Germany*

Tools Demo: RGD Pathway Tools

Neurosciences/Behavior/Disease
Chairpersons: B.E. Levin, *VA Medical Center, East Orange, New Jersey;* T. Serikawa, *Kyoto University, Japan*

Keynote Address
S. Pääbo, *Max-Planck Institute for Evolutionary Anthropology and Biology*

Manipulating the Genome
Chairpersons: Q.-L. Ying, *University of Southern California, Los Angeles;* E. Cuppen, *Hubrecht Institute, Utrecht, The Netherlands*

Systems Biology/Resources
Chairpersons: P. Flicek, *European Bioinformatics Institute, Hinxton, United Kingdom;* A. Kwitek, *University of Iowa, Iowa City*

Keynote Address
M. Mann, *Max-Planck Institute for Biochemistry*

Cardiovascular
Chairpersons: H. Jacob, *Medical College of Wisconsin, Milwaukee;* R. Kreutz, *Charité-Universitätsmedizin Berlin, Germany*

A. Voskresenskiy

M. Busch-Dienstfertig, M. Schwemmle

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 15–28

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

J. Cao, University of California, Los Angeles
M. Chambers, University of California, Los Angeles
R. Chumanov, University of Wisconsin, Madison
C.-F. Huang, M.D. Anderson Cancer Center, University of Texas, Houston
Y.-C., Lee, M.D. Anderson Cancer Center, University of Texas, Houston
S. Nechaev, NIEHS/Research Triangle Park, North Carolina
K. Pennington, Brandeis University, Waltham, Massachusetts
N. Thompson, University of Wisconsin, Madison
W. Turki-Judeh, University of California, Los Angeles

This course was for scientists unfamiliar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with a discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue, (2) a sequence-specific DNA-binding protein, (3) a recombinant protein overexpressed in *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 utilized to include immunological and biochemical assays, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Adams, S., Ph.D., Mayo Clinic Jacksonville, Florida
Arizgolshani, O., B.S., University of California, Los Angeles
Bartlow, P., B.S., University of Pittsburgh, Pittsburgh, Pennsylvania
Bellail, A., Ph.D., Emory University, Atlanta, Georgia
Broghammer, A., Dipl., University of Aarhus, Denmark
Catera, R., M.S., The Feinstein Institute for Medical Research, Manhasset, New York
Chen, E., Ph.D., National Institutes of Health, Bethesda, Maryland
Cherk, C., B.A., University of California, Berkeley
Hao, C., Ph.D., Emory University, Atlanta, Georgia
Knies, J., Ph.D., Brown University, Providence, Rhode Island
Lange, S., Ph.D., University of Texas M.D. Anderson Cancer Center, Science Park, Smithville
Lomenick, B., B.S., University of California, Los Angeles
Minaker, S., B.Sc., University of British Columbia, Vancouver, Canada
Shapiro, M., A.B., University of Texas Health Science Center, San Antonio
Stanciu, L., Ph.D., Purdue University, West Lafayette, Indiana
Toomey, E., B.S., University of Utah, Salt Lake City

SEMINARS

Adelman, K., NIEHS/Research Triangle Park, North Carolina: Stress-responsive genes are poised for activation.
Burgess, R., University of Wisconsin, Madison: Welcome and introduction to course. Introduction to protein purification. RNA polymerase–σ factor interactions and use of LRET-based assays for drug discovery and biochemistry.
Chumanov, R., University of Wisconsin, Madison: Halo-Tag and CARM1 purification.
Courey, A., University of California, Los Angeles: System-wide analyses of Groucho and SUMO in *Drosophila* development.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Why do prostate cancer cells go to the bone?
Marr, M., Brandeis University, Waltham, Massachusetts: Controlling gene expression in response to extracellular signals.
Nechaev, S., NIEHS/Research Triangle Park, North Carolina: Promoting proximal RNA Polymerase II Stalling Genome-wide.
Thompson, N., University of Wisconsin, Madison: Immunoaffinity chromatography.

Cell and Developmental Biology of *Xenopus*

April 17–28

INSTRUCTORS **R. Keller,** University of Virginia, Charlottesville
K. Kroll, Washington University School of Medicine, St. Louis, Missouri

ASSISTANTS **C. Flournoy,** University of Virginia, Charlottesville
S. Louie, University of Virginia, Charlottesville
P. Skoglund, 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. This course combined intensive laboratory training with daily lectures from recognized experts in the field. Students learned both emerging technologies and classical techniques to study gene function in *Xenopus* development. An important element was the informal interaction between students and course faculty.

This course was supported with funds provided by the National Institute of Child Health and Human Development, the National Science Foundation, and the Howard Hughes Medical Institute.

PARTICIPANTS

Belgacem, Y., Ph.D., University of California, Davis
Danilchik, M., Oregon Health & Science University, Portland
Dolde, C., Dipl., German Cancer Research Center, Heidelberg, Germany
Gentsch, G., M.A., University of Cambridge, United Kingdom
Hager, H., B.S., Vanderbilt University, Nashville, Tennessee
Immani, S., Ph.D., University of California, Davis
Lau, N., Ph.D., Massachusetts General Hospital, Boston
Møbjorg, N., Ph.D., University of Copenhagen, Denmark
Morales Diaz, H., B.S., Dana Farber Cancer Institute, Boston, Massachusetts
Nicetto, D., B.S., Ludwig-Maximilian Universitat, Munchen, Germany
Park, P., Ph.D., Case Western Reserve University, Cleveland, Ohio
San Miguel-Ruiz, J., B.S., University of Minnesota, Minneapolis
Singh, A., Ph.D., National Cancer Institute at Frederick, Frederick, Maryland
Sproul, A., Ph.D., Mount Sinai Medical School, New York
Thelie, A., Ph.D., Institut de Biologie et de Medecine Moleculaire, Charleroi, Germany
Watson, F., Ph.D., Washington & Lee University, Lexington, Virginia

SEMINARS

Amaya, E., University of Manchester, United Kingdom: Post-gastrula organogenesis.
Grainger, R., University of Virginia, Charlottesville: Genomic approaches for enhancer identification in *Xenopus*.
Harland, R., University of California, Berkeley: Biology of the organizer.
Hill, C., Cancer Research UK/London Research Institute, London, United Kingdom: TGF-β superfamily signaling in early *Xenopus* development.
Houston, D., University of Iowa, Iowa City: Roles of localized maternal mRNAs in *Xenopus* axis formation.
Keller, R., University of Virginia, Charlottesville: Morphogenesis.
Khokha, M., Yale University, New Haven, Connecticut: Introduction to *Xenopus tropicalis*.
Kroll, K., Washington University School of Medicine, St. Louis, Missouri, and Keller, R., University of Virginia, Charlottesville: Introduction to *Xenopus*.
Zimmerman, L., National Institute for Medical Research, London, United Kingdom: Genetics approaches in *Xenopus tropicalis*.
Zorn, A., Children's Hospital Medical Center, Cincinnati, Ohio: Mesendoderm and gut development.

Molecular Neurology and Neuropathology

June 3–9

INSTRUCTORS **M. Cookson,** National Institute on Aging/NIH, Bethesda, Maryland
B. Davidson, University of Iowa, Des Moines
H. Orr, University of Minnesota, Minneapolis

This intensive 1-week discussion course offered successful applicants a unique opportunity to learn the latest concepts and methodologies associated with the study of human neurological disorders such as Alzheimer's, Parkinson's, and epilepsy. Participants discussed in detail the strengths and weaknesses of the accumulated experimental evidence underlying our current understanding of these diseases. Fundamental questions, such as how and why particular neurons die in certain disorders, were discussed in the context of identifying the best experimental approaches to finding answers, whether through the use of transgenic and/or lesion-induced mouse models, functional brain and/or cellular imaging, gain/loss-of-function molecular and viral approaches, cellular transplantation, or a combination of these approaches. The course examined why many of these disorders share apparently common features—protein aggregation, specific vulnerability of certain classes of neuron, long incubation period—and discussed to what extent these features reflect common pathological mechanisms. Extended seminars and discussion by a wide range of leading investigators further illuminated developments in this rapidly moving field. Participation in the course provided an essential conceptual and methodological framework for anyone intending to pursue rigorous research.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Anaya, F., B.S., University College, London Institute of Neurology, United Kingdom
Bjorkblom, B., Ph.D., Stavanger University Hospital, Stavanger, Norway
Coombs, K., B.S., Arizona State University, Tempe
Gideons, E., B.S., National Institutes of Health, Bethesda, Maryland
Gray, S., Ph.D., University of North Carolina, Chapel Hill
Griffiths, L., B.S., University of Edinburgh, Edinburgh, United Kingdom
Hagan, C., Ph.D., University of Washington, Seattle
Hart, M., B.S., University of Pennsylvania, Philadelphia
Hemsley, K., Ph.D., Women's and Children's Hospital, South Adelaide, Australia
Krako, N., B.S., Scuola Normale Superiore, Pisa, Italy
McKee, M.A., M.D., Henry Ford Hospital, Ann Arbor, Michigan
Morales-Villagran, A., Ph.D., University of Guadalajara, Jalisco, Mexico
Pancrazi, L., Ph.D., Scuola Normale Superiore, Pisa, Italy
Panos, J., B.S., Western Michigan University, Kalamazoo
Plun-Favreau, H., Ph.D., University College, London Institute of Neurology, United Kingdom
Runko, A., Ph.D., National Institutes of Health, Bethesda, Maryland
Scali, M., B.S., Scuola Normale Superiore, Pisa, Italy
Thut, C., Ph.D., Merck Research Labs, West Point, Pennsylvania
White, M., Ph.D., University of Edinburgh, Edinburgh, United Kingdom

SEMINARS

Cookson, M., National Institute on Aging/NIH, Bethesda, Maryland: Parkinson's disease.
Davidson, B., University of Iowa, Des Moines: Polyglutamine and RNA.
Duff, K., Columbia University, New York: Models of Alzheimer's disease.
Han, M.H., Stanford University, Stanford, California: Multiple sclerosis.
Heikenwaelder, M., Institute for Neuropathology, Zurich, Switzerland: The molecular biology of prion diseases.
Iadecola, C., Cornell University, Ithaca, New York: Stroke.
McNamara, J., Duke University, Durham, North Carolina: Neurotrophins and epileptogenesis.
Morris, H., Cardiff University, Cardiff, United Kingdom: Neurological aspects.
Nelson, D., Baylor University, Houston, Texas: Fragile X/FTAS.
Orr, H., University of Minnesota, Minneapolis: Triplet repeat disorders.
Rademakers, R., Mayo Clinic College of Medicine, Jacksonville, Florida: Frontotemporal and other dementias.
Wong, P., Johns Hopkins University, Baltimore, Maryland: ALS.
Wynshaw-Boris, A., University of California, San Diego: Disorders of neuronal migration.
Vonsattel, J.P., Columbia University, New York: Neuropathology.

Advanced Bacterial Genetics

June 3–23

INSTRUCTORS **J. Kirby,** University of Iowa, Iowa City
S. Lovett, Brandeis University, Waltham, Massachusetts
A. Segall, San Diego State University, San Diego, California

ASSISTANTS **A. Cendrowski,** Brandeis University, Waltham, Massachusetts
M. Coleman, Massachusetts Institute of Technology, Cambridge
I. Naili, San Diego State University, San Diego, California
J. Willett, University of Iowa, Iowa City

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 polymerase chain reaction (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 and cloning methods; epitope insertion mutagenesis; site-directed mutagenesis; and fluorescence microscopy. Key components of the course were sophisticated genetic methods in the analysis of model bacteria (including *Escherichia coli*, *Salmonella, Bacillus subtilis*, and *Myxococcus xanthus*) and the use of a 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.

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.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Alexander, R., Ph.D., Yale University, New Haven, Connecticut
Atwal, M., Ph.D., Cold Spring Harbor Laboratory
Blackburn, M., B.S., Massachusetts Institute of Technology, Cambridge
Cha, J., Ph.D., New York University Medical Center, New York
Cimini, D., Ph.D., Second University of Naples, Naples, Italy
Gallego, A., B.S., Instituto de Biotecnologia, Cuernavaca, Mexico
Guida, B., B.S., Arizona State University, Tempe
Hargreaves, K., B.Sc., University of Leicester, United Kingdom
Hatahet, F., B.Sc., University of Oulu, Finland
Hove-Andersen, J., M.S., Technical University Denmark, Frederiksberg, Denmark
Hunt, D., Ph.D., University of Hawaii, Honolulu
Kannan, S., Ph.D., University of Arkansas for Medical Science, Little Rock
Neuvonen, J., M.S., University of Turku, Finland
Sermaswan, R., Ph.D., Khon Kaen University, Thailand
Singh, A., M.S., Emory University, Atlanta, Georgia
Teng, S.-W., B.S., Princeton University, Princeton, New Jersey

SEMINARS

Bernhardt, T., Harvard Medical School, Cambridge, Massachusetts: Genetic analysis of cell wall biogenesis and phage mediated bacteriolysis.
Dworkin, J., Columbia University, New York: Identification of a novel signaling pathway that responds to muropeptides.
Gital, Z., Princeton University, Princeton, New Jersey: Novel cytoskeletons and cytoskeletal regulators in *Caulobacter crescentus*.
Goulian, M., University of Pennsylvania, Philadelphia: Deciphering bacterial cell signaling circuits.
Gross, C., University of California, San Francisco: Envelope stress and synthetic biology.
Hughes, K., University of Utah, Salt Lake City: Flagellar synthesis.
Maloy, S., San Diego State University, San Diego, Calfornia: From chromosome rearrangements to vaccines.
Pogliano, K., University of California, San Diego, La Jolla: The mechanism of sporulation in *Bacillus subtilis*.

Ion Channel Physiology

June 3–23

INSTRUCTORS

B. Clark, University College London, United Kingdom
M. Farrant, University College London, United Kingdom
N. Golding, The University of Texas, Austin
A. Lee, University of Iowa, Iowa City
M. Nolan, University of Edinburgh, United Kingdom

ASSISTANTS

J. Davie, Max-Planck Institute for Medical Research, Heidelberg, Germany
P. Dodson, University of Edinburgh, Edinburgh, United Kingdom
A. Fink, Columbia University, New York
D. Garden, University of Edinburgh, Edinburgh, United Kingdom
J. Gruendermann, University College London, United Kingdom
K. Hansen, Emory University School of Medicine, Atlanta, Georgia
L. Kreiner, Emory University, Atlanta, Georgia
L. Scott, University of Texas, Austin
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 allow neurons to carry out their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitter at central and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, (3) respond to neuromodulators with changes in functional properties, and (4) are developmentally required and regulated. The research interests of guest lecturers reflect these areas of emphasis. The laboratory component of the course introduced students to electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice preparations. Different recording configurations were used (e.g., whole-cell, cell-free, and nucleated patches) to examine macroscopic or single-channel activity. Similarly, various methods of ligand and drug application were demonstrated. The advantages and disadvantages of each method, preparation, and recording technique were considered in relation to the specific scientific questions being asked. Admissions priority is given to students and postdocs with a demonstrated interest, specific plans, and a supportive environment to apply these techniques to a defined problem.

This course was supported by the Howard Hughes Medical Institute.

PARTICIPANTS

Anthony, T., Ph.D., California Institute of Technology, Pasadena
Bohlen, C., B.S., University of California, San Francisco
Collins, K., Ph.D., Yale University, New Haven, Connecticut
Gold, M., Ph.D., University of Washington, Seattle
Graves, A., B.A., Northwestern University, Evanston, Illinois
Kawate, T., M.A., Oregon Health & Science University, Portland
McCarthy, E., Ph.D., Yale School of Medicine, New Haven, Connecticut
Petzold, B., M.S., Stanford University, Stanford, California
Rowe, A., Ph.D., University of Texas, Austin
Taves, S., B.S., University of North Carolina, Chapel Hill
Vandenberg, A., B.S., University of California, San Francisco
Vecchia, D., M.S., University of Padova, Italy

SEMINARS

Armstrong, C., University of Pennsylvania, Philadelphia: Ion channels: Design and purpose.
Dudman, J., Janelia Farm Research Campus, Ashburn, Virginia: HCN channels.
Golding, N., University of Texas, Austin: Dendrites.
Hausser, M., University College London, United Kingdom: Synaptic integration.
Hille, B., University of Washington, Seattle: Voltage-gated channels.
Jones, M., University of Wisconsin, Madison: The simplest possible theory of allostery in the $GABA_A$ receptor.
Larsson, P., University of Miami, Miami, Florida: Structure and function of voltage-gated proton channels.
Lee, A., University of Iowa, Iowa City: Voltage-gated Ca^{2+} channel signaling complexes.
Mantegazza, M., Instituto Neurologico Besta/Inserm Avenir, Milan, Italy: Voltage-gated Na^+ channels and epilepsy.
Scanziani, M., University of California, San Diego, La Jolla: Microcircuits.
Schaefer, A., Max-Planck Institut für Medizinische Forschung, Heidelberg, Germany: From glutamate receptors to behavior.
Sjöström, J., University College London, United Kingdom: A dendritic switch for synaptic plasticity.
Soltesz, I., University of California, Irvine: Modulatory mechanisms, cannabinoids.
Traynelis, S., Emory University, Atlanta, Georgia: Control of NMDA receptor function.
von Gersdorff, H., Oregon Health & Science University, Portland: Quantal size and multiquantal release at a sensory synapse.

Molecular Embryology of the Mouse

June 3–23

INSTRUCTORS
K. Hadjantonakis, Sloan-Kettering Institute, New York
J. Rivera, University of Massachusetts Medical School, Worcester
L. Pevny, University of North Carolina, Chapel Hill

ASSISTANTS
G. Kwon, Sloan-Kettering Institute, New York
A. Piliszek, Sloan-Kettering Institute, New York
L. Williams, University of North Carolina Medical School, Chapel Hill
R. Williams, University of North Carolina, Chapel Hill

This intensive laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse development. Lectures provided the conceptual basis for contemporary research in mouse embryogenesis and organogenesis, whereas laboratory practicals provided extensive hands-on introduction to mouse embryo analysis. Experimental techniques 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 embryonic stem (ES) cell injection, and transgenesis by pronuclear microinjection. In addition, the practicals featured increased emphasis on phenotypic analysis of mutants, including techniques of histology, in situ hybridization, immunohistochemistry, skeletal preparation, organ culture, and tissue recombination. The course also introduced the generation of iPS cells (induced pluripotent cells) and time-lapse microscopy of early gastrulation embryos and organ cultures (eye, kidney, and gut).

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Bledau, A., M.S., Center for Regenerative Therapies, Dresden, Germany
Burda, J., B.S., Mayo Clinic, Rochester, Minnesota
Cerda-Esteban, N., Dipl., Max-Delbruck Center for Molecular Medicine, Berlin, Germany
Demidov, O., Ph.D., Institute of Molecular & Cell Biology, Singapore
Deuve, L.-J., Ph.D., Institut Pasteur, Paris, France
Domigan, C., B.S., University of California, Los Angeles
Dorr, K., B.S., University of North Carolina, Chapel Hill
Heronymus, T., B.A., Northeastern Ohio University College of Medicine, Rootstown
Hong, S.-J., Ph.D., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
Oddsson, A., M.S., Karolinska Institutet, Stockholm, Sweden
Rak-Raszewska, A., M.Sc., University of Liverpool, United Kingdom
Sanchez Manuel, M., B.S., University of Salamanca, Spain
Schmerr, M., Ph.D., Cleveland Clinic, Cleveland, Ohio
Szypowska, A., M.Sc., Universitair Medisch Centrum Utrecht, The Netherlands

SEMINARS

Anderson, K., Sloan-Kettering Institute, New York: Hedgehog signaling, cilia, and development.
Anton, E., University of North Carolina School of Medicine, Chapel Hill: Cortical development and radial glial/neural stem cells.
Behringer, R., University of Texas M.D. Anderson Cancer Center, Houston: Generation and analysis of transgenic mice.
Capel, B., Duke University Medical Center, Durham, North Carolina: Germ cells, genital ridges, and sex differentiation.
Collignon, J., Institut Jacques Monod, Paris, France: TGF-β signaling in early embryo development.
Conlon, F., University of North Carolina, Chapel Hill: Heart development.
Costantini, F., Columbia University, College of Physicians & Surgeons, New York: Kidney organogenesis.
Dickinson, M., Baylor College of Medicine, Houston, Texas: Imaging cardiovascular function in mouse development and tissue engineering.
Eglis, M., Columbia University, New York: Nuclear reprogramming.
Hadjantonakis, K., Sloan-Kettering Institute, New York: Imaging cell behavior in early mouse embryos.
Hogan, B., Duke University Medical Center, Durham, North Carolina: Lung organogenesis.
Huppert, S., Vanderbilt University, Nashville, Tennessee: Liver organogenesis and regulation of three-dimensional intrahepatic bile duct formation/maintenance.
Johnson, R., M.D. Anderson Cancer Center, Houston, Texas: Limb development.
Joyner, A., Memorial Sloan-Kettering Cancer Center, New York: Patterning and cell fate mapping of the nervous system.
Justice, M., Baylor College of Medicine, Houston, Texas: Mouse ENU mutagenesis: From genomics to genetic modifiers of disease.
Kenney, A., Memorial Sloan-Kettering Cancer Center, New York: Hedgehog signaling in cerebellar development and meduloblastomas.
Lovell-Badge, R., MRC National Institute for Medical Research, London, United Kingdom: Sry, sex determination, and *sox* genes.
Magnuson, T., University of North Carolina, Chapel Hill: Epigenetics.
Nagy, A., Samuel Lunenfeld Research Institute, Toronto, Canada: Molecular genetics of pluripotent cells in the mouse.
Pevny, L., University of North Carolina, Chapel Hill: Retinogenesis.
Rivera, J., University of Massachusetts Medical School, Worcester: Lineage analysis and early postimplantation.
Rossant, J., The Hospital for Sick Children, Toronto, Canada: Chimeras, ES cell lineages.
Shen, M., Columbia University Medical Center, New York: Prostate development.
Solter, D., Institute of Medical Biology, Singapore: Preimplantation development.
Soriano, P., Mount Sinai School of Medicine, New York: Gene traps and PDGF/EPH/ephrin signaling.
Stewart, C., National Cancer Institute, Frederick, Maryland: ES cells, mouse strains.
Takahashi, J., HHMI/Northwestern University, Evanston, Illinois: Circadian rhythms.
Tam, P., Children's Medical Research Institute, Sydney, Australia: Gastrulation.
Threadgill D., University of North Carolina, Chapel Hill: Welcome, introductions, and overview. One hundred years of the mouse in research.
Trainor, P., Stowers Institute for Medical Research, Kansas City, Missouri: Craniofacial development disease and evolution.

Integrative Statistical Analysis of Genome-scale Data

June 8–23

INSTRUCTORS
H. Bussemaker, Columbia University, New York
V. Carey, Harvard University, Boston, Massachusetts
C. Leslie, Memorial Sloan-Kettering Cancer Center, New York
M. Reimers, Virginia Commonwealth University, Richmond

ASSISTANTS
M. Fazalollahi, Columbia University, New York
M. Figueroa, Cornell University, Ithaca, New York
E. Lee, Columbia University, New York
P. Palta, University of Tartu, Estonia
T. Riley, Rutgers University, Piscataway, New Jersey
M. Waisberg, National Institutes of Health/NIAID, Bethesda, Maryland

The 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 are listed on the following page.

- 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
- 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 human. Students were assumed to have a basic familiarity with the R programming language at the start of the course.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Benito, E., B.S., Institute for Neurosciences in Alicante, Spain, San Juan de Alicante, Spain
Biddie, S., B.Sc., National Institutes of Health, Bethesda, Maryland
Buchanan, G., Ph.D., University of Adelaide, Australia
Chao, M., B.A., Stanford University School of Medicine, Palo Alto, California
Chen, D., Ph.D., University of Vienna, Austria
Cui, Y., B.S., University of Kansas Medical Center, Kansas City
Della Gatta, G., Ph.D., Columbia University, New York
Duan, Q., Ph.D., Brigham & Women's Hospital, Boston, Massachusetts
Eveland, A., Ph.D., Cold Spring Harbor Laboratory
Fazzio, T., Ph.D., University of California, San Francisco
Greiner, D., M.D., Baylor College of Medicine, Houston, Texas
Gupta, A., M.S., University of Maryland/NIH, Bethesda
Imam, F., Ph.D., Harvard University, Cambridge, Massachusetts
Jima, D., M.S., Duke University Medical Center, Durham, North Carolina
Khankhanian, P., B.A., University of California, San Francisco
Lenstra, T., M.Sc., University Medical Center, Utrecht, The Netherlands
Leshkowitz, D., Ph.D., Weizmann Institute of Science, Rehovot, Israel
Lidschreiber, M., M.Sc., Munich University, Germany
Liu, X., M.S., University of Maryland Institute for Genome Science, Baltimore
Mukherjee, S., M.S., Gerstner Sloan-Kettering Graduate School, New York
Roessler, S., Ph.D., National Cancer Institute/NIH, Bethesda, Maryland
Tagne, J.-B., Ph.D., Boston University School of Medicine, Boston, Massachusetts
Tang, W., Ph.D., National Cancer Institute, Bethesda, Maryland
Wu, X., M.D., Ohio State University, Columbus
Yang, M., B.S., National Institutes of Health/NHGRI, Rockville, Maryland
Zamudio, J., B.S., Massachusetts Institute of Technology, Cambridge

SEMINARS

Bekiranov, S., University of Virginia, Charlottesville: Intro to oligo arrays and second-generation sequencing technology. Physics of hybridization.
Bulyk, M., Brigham & Womens Hospital and Harvard Medical School, Boston, Massachusetts: Protein binding microarrays I. Protein binding microarrays II.
Bussemaker, H., Columbia University, New York: Gene-set-based analysis. Representations of protein–DNA-binding specificity. Methods for de novo motif discovery. Linear models of transcriptional regulation I. Linear models of transcriptional regulation II. Overview of methods for predicting expression from sequence.
Carey, V., Harvard University, Boston, Massachusetts: Bioconductor/R for analysis of genome-scale data.
Culhane, A., Dana Farber Cancer Institute, Harvard, Cambridge, Massachusetts: Ordination.
Darnell, R., HHMI/The Rockefeller University, New York: Mapping protein–RNA interactions using HT sequencing II.

Futcher, B., Stony Brook University, Stony Brook, New York: Lessons from early microarray studies.
Irizarry, R., Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland: Affymetrix preprocessing: RMA, GCRMA, fRMA. Contemporary microarray applications: Genotyping, copy-number estimation (emphasis on batch effects), and whole-genome DNA. Methylation measurements. Sequencing base calling and quality assessment.
Kliebenstein, D., University of California, Davis: Quantitative genetics I. Quantitative genetics II.
Leslie, C., Memorial Sloan-Kettering Cancer Center, New York: Regulation by microRNAs. Intro to machine learning. Cancer classification.
Liu, S., Dana Farber Cancer Institute, Harvard University, Boston, Massachusetts: Analyzing ChIP-chip data. Analyzing ChIP-Seq data/epigenetics.
Lucito, R., Cold Spring Harbor Laboratory: Epigenetics and cancer.
Reimers, M., Virginia Commonwealth University, Richmond: Array CGH and ChIP-chip methods.
Zhang, M., Cold Spring Harbor Laboratory: Epigenetics.

Workshop on Autism Spectrum Disorders

June 19–26

INSTRUCTORS **D. Geschwind,** University of California, Los Angeles
P. Levitt, Vanderbilt University, Nashville, Tennessee
S. Spence, National Institute of Mental Health, Bethesda, Maryland

ASSISTANT **B. Bill,** University of California, Los Angeles

Autism spectrum disorders (ASDs) are developmental disorders with complex phenotypes defined by a triad of symptoms that include disrupted social abilities, verbal and nonverbal communication skills, and restricted interests with repetitive behaviors. Co-occurring neurological and medical conditions are not unusual in these disorders. The underlying etiology remains a mystery, but ASDs are the most highly heritable of neuropsychiatric disorders. This Workshop examined dimensions of ASD on various levels, including sessions on characteristics of the clinical syndrome; the neuropathology, imaging, and cognitive neuroscience studies that implicate circuits and systems involved in ASD; the current state of findings from human genetics; concepts regarding the developmental neurobiological basis of ASD; the use of experimental models; and current etiological theories and hypotheses of ASD.

In addition to hearing about the most recent research in these areas, we explored and debated controversial topics and challenges to basic assumptions in the field. An exceptional faculty with

diverse interests 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 build the foundation for their future research, it introduced them to many potential collaborators from different disciplines working to understand ASD.

This course was supported with funds provided by Autism Speaks, the Nancy Lurie Marks Family Foundation, and the Simons Foundation.

PARTICIPANTS

Ben Shalom, D., Ph.D., Ben-Gurion University, Be-er-Sheva, Israel
Ciaramitaro, V., Ph.D., University of California, San Diego/The Salk Institute for Biological Studies, La Jolla, California
D'Cruz, A.-M., B.S., University of Illinois, Chicago
Esposito, G., Ph.D., University of Trento, Rovereto TN, Italy
Florentini, C., B.S., University of Geneva, Geneva, Switzerland
Harony, H., Ph.D., University of Haifa, Haifa, Israel
Jacob, S., M.D., University of Illinois, Chicago
Johnston, P., B.S., Institute of Psychiatry, King's College London, United Kingdom
Kilpinen, H., B.S., Institute for Molecular Medicine, Helsinki, Finland
Lopatina, O., Ph.D., Kanazawa University Graduate School of Medicine, Kanazawa, Ishikawa, Japan
Lowe, J.K., Ph.D., University of California, Los Angeles
Millman, F., B.S., Curtin University of Technology, Perth, Australia
Oberman, L., Ph.D., Beth Israel Deaconess Medical Center, Boston, Massachusetts
Percaccio, C.R., Ph.D., University of Washington, Seattle
Philip, R., Ph.D., University of Edinburgh, Lothian, United Kingdom
Rudie, J., B.S., University of California/David Geffen School of Medicine, Los Angeles
Santos, R., B.S., University of California, Irvine
Stanfield, A., Ph.D., University of Edinburgh, Edinburgh, Midlothian, United Kingdom
Strathearn, L., M.D., Baylor College of Medicine, Houston, Texas
Wilson, E., B.S., Macquarie University, Sydney, Australia
Zaki, J., B.S., Columbia University, New York
Zinkstock, J., M.D., Institute of Psychiatry, King's College London, United Kingdom

SEMINARS

Biederer, T., Yale University, New Haven, Connecticut: Synaptic differentiation and autism spectrum disorders.
Fombonne, E., McGill University, Montreal, Canada: Epidemiology of ASD.
Geschwind, D., University of California, Los Angeles/Matt State/Yale University: Introduction to human genetics. Genetic findings in ASD: Common variants, rare mutations.
Geschwind, D., University of California, Los Angeles/Matt State/Yale University and Bill, B., University of California, Los Angeles: Genomic tools exercises: Bioformalities, database access, data gathering, and uses.
Hensch, T., Riken Institute, Japan: Critical periods/plasticity mechanisms.
Kasari, C., University of California, Los Angeles: Treatments overview.
Levitt, P., Vanderbilt University, Nashville, Tennessee: Neuroanatomical systems implicated in ASD and neuropathology. Introduction to developmental neurobiology. Neurodevelopmental process in ASD.
Martinez, F., University of Arizona, Tucson: Environmental contributions: Asthma as a model.
Minshew, N., University of Pittsburgh, Pennsylvania: Adults with autism.
Murphy, D., Institute of Psychiatry, Kings College: Neuroimaging findings in ASD.
Nelson, C., Harvard University, Boston, Massachusetts: Introduction to imaging and ERP. Neurocognition and face processing mechanisms.
Pardo-Villamizar, C., Johns Hopkins University School of Medicine, Baltimore, Maryland: Neuroimmunology.
Paylor R., Baylor College of Medicine, Houston, Texas: Mammalian animal models.
Rapin, I., Albert Einstein School of Medicine, Bronx, New York: History of ASD.
Rogers, J., Baylor College of Medicine, Houston, Texas: Evolutionary perspectives.
Sebat, J., Cold Spring Harbor Laboratory: Chromosomal abnormalities/syndromes.
Spence, S., National Institute for Mental Health, Bethesda, Maryland: Clinical assessments (clinical videos, student exercises: ADI-R, ADOS, SRS). Medical models/comorbidities. Endophenotypes-language delay, epilepsy/regression, OFC.
Szyf, M., McGill University, Montreal, Canada: Epigenetics.
Zwaigenbaum, L., University of Alberta, Edmonton, Canada: Clinical presentation, core features. ASD measures, broader phenotypes, phenotypic variability, outcomes.
Special guest: Portia Iversen, author of "Strange Son."

Computational Cell Biology

June 26–July 16

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 & Mary, Williamsburg, Virginia
J. Tyson, Virginia Polytechnic Institute, Blacksburg

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, of programmed cell death, of mitotic chromosome movements, and of synthetic gene networks. Unlike computational genomics or bioinformatics, computational cell biology focuses 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 lectures on the mathematics of dynamical systems, computational simulation techniques, cell biology, and molecular biology. Practicing theoreticians and experimentalists rotated 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.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Barik, D., Ph.D., Virginia Polytechnic Institute and State University, Blacksburg
Borinskaya, S., B.S., University of Connecticut Health Center, Farmington
Braga, J., Ph.D., Instituto de Medicina, Lisboa, Portugal
Brown, S.-A., M.A./B.A., University of Connecticut Health Center, Farmington
D'Ausillo, C., B.S., Dartmouth College, Hanover, New Hampshire
Fai, T., B.S., New York University, New York
Fant, A., B.S., University of North Carolina, Chapel Hill
Fertig, E., Ph.D., Johns Hopkins University, Baltimore, Maryland
Frick, P., B.S., Vanderbilt University, Nashville, Tennessee
Gomez-Cabrero, D., Ph.D., Clinical Gene Networks AB, Stockholm, Sweden
Gottstein, W., M.S., Institute for Theoretical Biology, Berlin, Germany
Huang, W., B.S., The Huck Institutes of Life Sciences, University Park, Pennsylvania
Hunziker, A., M.S., Niels Bohr Institute, Copenhagen, Denmark
Kang, Q., B.E., Indiana University, Bloomington
LaMar, M., Ph.D., The College of William & Mary, Williamsburg, Virginia
McDougal, R., B.S., The Ohio State University, Columbus
Mifsud, W., M.D., University of Cambridge, United Kingdom
Ogasawara, H., Ph.D., ATR, Kyoto, Japan
Pang, T.Y., B.S., SUNY, Stony Brook, New York
Petznick, G., M.Sc., University of Applied Sciences Wildau, Germany
Purcell, O., M.Sc., University of Bristol, United Kingdom
Razinkov, I., B.S., University of California, San Diego
Siegal-Gaskins, D., Ph.D., Ohio State University, Columbus
Soneral, P., Ph.D., Bethel University, St. Paul, Minnesota
Williams, C., Ph.D., North Carolina State University, Raleigh
Wuttke, A., B.S., Uppsala University, Sweden
Xu, B., B.S., Princeton University, Princeton, New Jersey

SEMINARS

Bhalla, U., National Center for Biological Sciences, Bangalore, India: Research results: Neuronal signaling–Electricity meets chemistry. Research results: Switches and memory.
Blackwell, A., George Mason University Krasnow Institute, Fairfax, Virginia: Background and concepts: Signaling pathways in synaptic plasticity and learning. Research results: The role of the basal ganglia in learning.
Civelekoglu-Scholey, G., University of California, Davis: Background and concepts: Modeling mitosis–How dynamic microtubules and mitotic motors cooperate to segregate the genome. Research results: Modeling mitosis–How dynamic microtubules and mitotic motors cooperate to segregate the genome.
Cytrynbaum, E., University of British Columbia, Vancouver, Canada: Didactic series–Stochastic models of polymers. Didactic series–Stochastic models of molecular motors. Background and concepts–Deterministic and stochastic models of polymer dynamics in bacterial cell division. Research results–Deterministic and stochastic models of polymer dynamics in bacterial cell division.
Dupont, G., Universite Libre de Bruxelles, Brussels, Belgium: Background and concepts: Minimal model for Ca^{2+}. Research results: Modeling calcium signaling.
Elston, T., University of North Carolina, Chapel Hill: Background and concepts: Modeling simple gene networks. Research results: Modeling signal transduction in yeast.
Ermentrout, G.B., University of Pittsburgh, Pennsylvania: Didactic series–I, Planar systems of differential equations. Didactic series–II, Simple biophysical models for excitability. Introduction to dynamical systems using XPP.
Fall, C., University of Illinois, Chicago: Didactic series: Scope of cellular dynamics.
Hasty, J., University of California, San Diego, La Jolla: Background and concepts: Engineered gene networks–Variability and oscillations. Research results: Engineered gene networks–Variability and oscillations.
Haugh, J., North Carolina State University, Raleigh: Background and concepts: Quantitative cell biology–Integrating experiments and modeling. Research results: Analysis of intracellular signaling driven by cell population and live cell microscopy data.
Hucka, M., California Institute of Technology, Pasadena: SBML, BioModels database, and related resources.
Iyengar, R., Mt. Sinai School of Medicine, New York: Network analysis: Getting the big picture. Integrating network analyses and differential equation-based models.

Keener, J., University of Utah, Salt Lake City: Cardiac cells and excitability. Cardiac arrhythmias: Excitability in spatially extended systems.

Lahav, G., Harvard Medical School, Boston, Massachusetts: Background and concepts: The DNA-damage signaling network. Research results: Studying p53's dynamics in single cells.

Lechleiter, J., University of Texas Health Sciences Center, San Antonio: Background and concepts: Biology of calcium signaling. Research results: Biology of calcium signaling.

Loew, L., University of Connecticut Health Center, Farmington: Background and concepts: Dendritic nucleation model. Research results: Dendritic nucleation model.

Mendes, P., University of Manchester School of Computer Science, Manchester, United Kingdom: Parameter estimation using COPAS1.

Moraru, I., University of Connecticut Health Center, Farmington: Overview of virtual cell and the concept of cell physiology.

Peskin, C., New York University, New York: Computation of blood flow in the heart by the immersed boundary method. Generalizations of the immersed boundary method for cardiac electrophysiology, the dynamics of bent twisted filaments in fluid, and fluid–structure interaction with Brownian motion.

Phair, R., Integrative Bioinformatics, Los Altos, California: A revisionist model of protein and lipid traffic in the Golgi apparatus. A scientific consultant's idealistic but passionate view of CCB.

Pomerening, J., Indiana University, Bloomington: Didactic series–Molecular and physiological principles of cell cycle regulation. Research results: Cell cycle control systems–Bistability and oscillations in the *Xenopus* early embryo. Didactic series–Positive feedback as a recurring theme in signaling systems. Didactic series–Fusing computational and experimental approaches to uncover systems-level signaling behaviors.

Schaff, J., University of Connecticut Health Center, Farmington: Spatial modeling using virtual cell.

Sherman, A., National Institutes of Health/NIDDK, Bethesda, Maryland: ER vs. plasma membrane drive calcium oscillations. Metabolically regulated and driven calcium oscillations.

Slepchenko, B., University of Connecticut Health Center, Farmington: Compartmental modeling.

Smith, G., The College of William & Mary, Williamsburg, Virginia: Didactic series–Cellular biophysics and ODEs. Didactic series–Random walks and diffusion. Didactic series–Reaction-diffusion and advection. Didactic series–Markov chain models of ion channels. Didactic series–Deterministic and stochastic reaction networks. Background and concepts–Markov chain models and calcium release sites. Research results–Moment closure for local control of calcium-induced calcium release in cardiac myocytes.

Terman, D., Ohio State University, Columbus: Didactic series–Nonlinear dynamics review and new concepts. Didactic series–Waves in biology. Didactic series–Fast and slow time scales.

Tyson, J. and Barik, D., Virginia Polytechnic Institute and State University, Blacksburg: Didactic series–How do cells compute? Didactic series–Network motifs and functional modules in macromolecular regulatory systems. Didactic series–Design principles of biochemical oscillators. Didactic series–Past, present, and future computational biology.

Advanced Techniques in Plant Science

June 26–July 16

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. Lee,** University of Massachusetts, Amherst
M. Pieck, Boston University, Boston, Massachusetts
L. Wang, Cornell University, Ithaca, New York

This course provided an intensive overview of topics in plant physiology, biochemistry, and development, focusing on molecular genetic and analytical approaches to understand plant biology. It emphasized recent results from *Arabidopsis*, maize, and a variety of other plants and provided an introduction to current methods used in plant molecular biology. It was designed for scientists with some experience in molecular techniques or plant biology who wish to work with plants using the latest technologies in genomics, 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 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 address current agronomic problems. Lectures describing bioinformatics tools available to the plant community and the resources provided by plant genome projects were also included. Speakers provided overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions provided an introduction to important techniques currently used in plant research. These included studies of

plant development, mutant analysis, histochemical staining, transient gene expression, gene silencing, applications of fluorescent protein fusions, protein interaction and detection, proteomics approaches, several different approaches for quantifying metabolites, transient transformation, and techniques commonly used in genetic and physical mapping. The course also included several short workshops on important themes in plant research.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Borges, F., Lic., Instituto Gulbenkian de Ciencia, Oeiras, Portugal
Brondani, C., Ph.D., Brazilian Organization for Agriculture Research, Madison, Wisconsin
Christiansen, M., M.S., Aarhus University, Slagelse, Denmark
Fiil, B., M.S., University of Copenhagen, Denmark
Goodspeed, D., B.S., Rice University, Houston, Texas
Horner, W., B.S., Syngenta Biotechnology, Inc., Research Triangle Park, North Carolina
Liu, J., B.S., Rice University, Houston, Texas
Liu, X., B.S., University of Minnesota, St. Paul
Powikrowska, M., Ms.C., University of Copenhagen, Denmark
Toyokura, K., M.S., National Institute for Basic Biology, Okazaki, Japan
Van Houtte, H., M.S., VIB K.U. Leuven, Heverlee, Belgium
Wendell, M., M.S., Norwegian University of Life Sciences, Aas, Norway
Winter, N., B.S., University of Vienna, Austria
Zhang, P., B.S., University of Southern California, Los Angeles

SEMINARS

Brutnell, T., Boyce Thompson Institute, Ithaca, New York: Plastids.
Celenza, J., Boston University, Boston, Massachusetts: Secondary metabolites: Glucosinolates.
Dinesh-Kumar, S., Yale University, New Haven, Connecticut: Plant pathogen interactions.
Folta, K., University of Florida, Gainesville: Strawberry genomics.
Grotewold, E., University of Ohio, Columbus: Regulatory networks.
Harmer, S., University of California, Davis: Circadian rhythms.
Harmer, S., University of California, Davis and Kellogg, E., University of Missouri, St. Louis: iPlant.
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.
Kim, H.-J., Dartmouth College, Hanover, New Hampshire: Transient transformation and analysis using plant protoplasts.
Kuhlemeier, C., University of Bern, Switzerland: Phyllotaxis.
Maloof, J., University of California, Davis: Light regulation.
Morohashi, K., Ohio State University, Columbus: ChIP and ChIP-chip approaches to establish plant regulatory motifs.
Normanly, J., University of Massachusetts, Amherst: Metabolomics.
Nuhse, T., University of Manchester, United Kingdom: Proteomics.
Paszkowski, U., Universite de Lausanne, Switzerland: Root development and physiology.
Rose, J., Cornell University, Ithaca, New York: Cell walls.
Schaller, G.E., Dartmouth College, Hanover, New Hampshire: Hormone signaling.
Slotnik, K. and Tanurdzic, M., Cold Spring Harbor Laboratory: Epigenetics.
Sussex, I., Yale University, New Haven, Connecticut: Introduction to plant structure.
Timmermans, M., Cold Spring Harbor Laboratory: microRNA regulation.
Vogel, J., Western Regional Research Center, USDA-ARS, Albany, California: Feedstock development for biofuels.
Wang, X., University of Missouri, St. Louis: Lipid signaling.

Neurobiology of *Drosophila*

June 26–July 16

INSTRUCTORS **R. Allada,** Northwestern University, Evanston, Illinois
H. Broihier, Case Western Reserve University, Cleveland, Ohio
D.E. Featherstone, University of Illinois, Chicago

ASSISTANTS **K. Chen,** University of Illinois, Chicago
B. Chung, Northwestern University, Evanston, Illinois
V. Kilman, Northwestern University, Evanston, Illinois
I. Nechipurenko, Case Western Reserve University, Cleveland, Ohio
C. Miller, Case Western Reserve University, Cleveland, Ohio

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students through established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The 3-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and directed laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that are used in the investigation of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition

to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques including embryo in situ hybridization, labeling of identified neurons, electrophysiology (intracellular recording and voltage clamping), optical imaging of neuronal and cellular 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 course was supported with funds provided by the National Institute of Mental Health, the National Institute of Neurological Disorders & Stroke, and the Howard Hughes Medical Institute.

PARTICIPANTS

Cho, R., Ph.D., Massachusetts Institute of Technology, Cambridge
Dean, T., M.S., University of Pennsylvania/School of Medicine, Philadelphia
Gomez-Marin, A., Ph.D., Centre for Genomic Regulation, Barcelona, Spain
Johnson, O., B.S., Louisiana State University Health Sciences Center, New Orleans
Kozhevnikova, E., M.S., Erasmus Medical Center, Rotterdam, Germany
Linford, N., Ph.D., Baylor College of Medicine, Houston, Texas
McLear, J., Ph.D., Utica College, Utica, New York
Samuel, A., Ph.D., Harvard University, Cambridge, Massachusetts
Shirangi, T., Ph.D., HHMI/University of Wisconsin, Madison
Xiong, X., B.S., University of Michigan, Ann Arbor
Yi, C., A.B., Harvard Medical School, Boston, Massachusetts
Yuva, A.Y., B.S., University of Muenster, Germany

SEMINARS

Allada, R., Northwestern University, Evanston, Illinois: Circadian rhythms and sleep.
Benton, R., University of Lausanne, Switzerland: Olfaction.
Broihier, H., Case Western University, Cleveland, Ohio: Fly genetics 101. Motorneurons.
Ejima, A,, Brandeis University, Waltham, Massachusetts: Courtship and learning.
Freeman, M., University of Massachusetts, Worcester: Glia. Bioinformatics/web tools.
Goodwin, S., Glasgow University, Glasgow, United Kingdom: Courtship and learning.
Grueber, W., Columbia University, New York: Dendrites.
Jefferis, G., Cambridge University, Cambridge, United Kingdom: Olfactory development. Mosaic techniques.
Kernan, M., SUNY, Stony Brook, New York: Mechanotransduction and hearing.
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, Pittsburgh, Pennsylvania: Imaging.
Lueven, P., VIB and K.U. Leuven, Belgium: Neurotransmitter release, vesicle cycling, and acute protein inactivation.
Montell, C., Johns Hopkins University, Baltimore, Maryland: Vision, taste, sensory transduction.
O'Connor-Giles, K., University of Wisconsin, Madison: NMJ structure and development.
O'Dowd, D., University of California, Irvine: Channels and membrane properties.
Rothenfluh, A., University of Texas Southwestern, Dallas: Flies and drugs.
Sigrist, S., European Neuroscience Institute Gottingen, Germany: Protein tagging, live tracking, and nanoimaging.
Turner, G., Cold Spring Harbor Laboratory: Research talk and patch demo.
van Swinderen, B., University of Queensland, Australia and Clandinin, T., Stanford University, Palo Alto, California: Attention/visual behavior.
Waddell, S., University of Massachusetts, Worcester and Dubnau, J., Cold Spring Harbor Laboratory: Learning and memory.

Advanced Techniques in Molecular Neuroscience

June 30–July 16

INSTRUCTORS
C. Lai, Indiana University, Bloomington
R. Lansford, California Institute of Technology, Pasadena
B. Stevens, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts

ASSISTANTS
T. Bell, University of Pennsylvania, Philadelphia
C. Canaria, California Institute of Technology, Pasadena
C. Challis, Indiana University, Bloomington
S. Gattu, Indiana University, Bloomington
V. Gradinaru, Stanford University, Stanford, California
D. Huss, California Institute of Technology, Pasadena
D. Schafer, Children's Hospital, Boston, Massachusetts

This newly revised laboratory and lecture course is designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the most advanced techniques in molecular neuroscience. The course curriculum was divided into three sections: an extensive and up-to-date set of laboratory exercises, daily lectures covering the theoretical and practical aspects of the various methods used in the laboratory, and a series of evening research seminars. The informal and interactive evening lectures were given by leading molecular neuroscientists and served to illustrate the ways in which the various experimental approaches have been used to advance specific areas of neurobiology. In this year's course, the laboratory portion included topics such as an introduction to the design and use of animal virus vectors in neurobiology; the use of small interfering RNAs

(siRNA) for regulating the expression of specific genes in neurons; practical exercises in gene-delivery systems including mammalian cell transfection protocols and single-cell electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use, and design of bacterial artificial chromosome (BAC) transgenic vectors; multiplex and whole-genome expression analyses using the most recent DNA microarray technologies (including labeled probe preparation, data analyses, mining, and interpretation); quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR) analyses from small numbers of cells (RNA purification, PCR optimization, interpretation of results); single-cell PCR and cDNA library construction; 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.

This course was supported with funds provided by the National Institute of Mental Health, the National Institute of Neurological Disorders & Stroke, and the Howard Hughes Medical Institute.

PARTICIPANTS

Abel, J., Ph.D., University of Virginia Medical School, Charlottesville
Biedenkapp, J., M.A., Massachusetts Institute of Technology, Cambridge
Boije, H., Ph.D., Uppsala University, Sweden
Chetty, S., B.A., University of Berkeley, California
Foo, L., B.Sc., Stanford University, Stanford, California
Gulacsi, A., Ph.D., The Rockefeller University, New York
Hinman, M., B.A., Case Western Reserve University, Cleveland, Ohio
Joseph-de Saram, P., M.Phys., Princeton University, Princeton, New Jersey
Leak, R., Ph.D., University of Pittsburgh, Pennsylvania
Leao, R., Ph.D., Karolinska Institute, Stockholm, Sweden
Liang, W., Ph.D., Translational Genomics Research Institute, Phoenix, Arizona
Mainardi, M., B.S., Scuola Normale Superiore, Pisa, Italy
Phillips, M., B.S., University of Houston, Houston, Texas
Stress, M., M.S., Max-Planck Institute of Neurobiology, Martinsried, Germany
Weishhana, K., Ph.D., Emory University School of Medicine, Atlanta, Georgia
Young, C., M.Sc., University of Calgary, Canada

SEMINARS

Barres, B., Stanford University School of Medicine, Stanford, California: Neuron–glial interactions in the CNS.
Darnell, R., The Rockefeller University, New York: Decoding RNA maps in the brain.
Eberwine, J., University of Pennsylvania Medical School, Philadelphia: Metastability of the genome–Impact on neuronal phenotype and dendritic biology.
Fishell, G., New York University Medical School, New York: Making up your mind: The developmental and genetic origins of cortical interneurons.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Connectomics.
Lipscombe, D., Brown University, Providence, Rhode Island: Neuronal calcium channels: Spliced for optimal function.
Lledo, P.-M., Institut Pasteur, Paris, France: Development of neural stem cells in adult circuits.
McCombie, W., Cold Spring Harbor Laboratory: Introduction to genomic analysis.
Reiter, J., University of California, San Francisco: The cell biology of signaling: Primary cilia interpret Hedgehog cues in development and disease.

Structure, Function, and Development of the Visual System

July 1–14

INSTRUCTORS **B. Chapman,** University of California, Davis
W.M. Usrey, University of California, Davis

ASSISTANT **C. Speer,** Ph.D., University of California, Davis

This lecture/discussion course explored the functional organization and development of the visual system as revealed by the use of a variety of anatomical, physiological, and behavioral methods. It was designed for graduate students and more advanced researchers who wish to gain a basic understanding of the biological basis for vision and to share in the excitement of the latest developments in this field. Topics included phototransduction and neural processing in the retina; functional architecture of striate cortex; cellular basis of cortical receptive field properties; the anatomy, physiology, and perceptual significance of parallel pathways; functional parcellation of extrastriate cortex; the role of patterned neuronal activity in the development of central visual pathways; and molecular mechanisms of development and plasticity in the visual system.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Ashourvan, A., Ph.D., School of Optometry, Bloomington, Indiana
Baker, L., B.S., Colorado State University, Fort Collins
Cerri, C., B.S., Scuola Normale Superiore of Pisa, Italy
Day-Brown, J., B.S., University of Louisville, Louisville, Kentucky
Failor, S., B.S., Center for Neuroscience, University of California, Davis
Hansen, B., B.S., University of Texas Health Science Center, Houston
Lee, S.-H., Ph.D., University of California, Berkeley
Manno, I., B.S., Scuola Normale Superiore of Pisa, Italy
Mayo, J.P., B.S., University of Pittsburgh, Pittsburgh, Pennsylvania
Okazaki, Y., B.S., Graduate School of Frontier Biosciences, Osaka, Japan
Otero-Millan, J., B.S., Barrow Neurological Institute, Phoenix, Arizona
Pietrasanta, M., B.S., Scuola Normale Superiore of Pisa, Italy
Ringuette, R., B.S., University of Ottawa, Ottawa, Canada
Triplett, J., Ph.D., University of California, Santa Cruz
Tsirlin, I., B.S., York University, Center for Vision Research, Toronto, Canada
Wagner-Schuman, M., B.S., Medical College of Wisconsin, Milwaukee
Whiting, R., B.S., University of Missouri, Columbia
Wright, T., Grad., Hospital for Sick Children, Gothenburg University, Toronto, Canada
Zeiner, K., B.S., University of St. Andrews, St. Andrews, United Kingdom

SEMINARS

Berson, D., Brown University, Providence, Rhode Island: Retina 1.
Brose, K., Cell Press, Cambridge, Massachusetts: Behind the scenes of the review process at neuron.
Chapman, B., University of California, Davis: How the visual system got its stripes.
Chichlilnisky, E.J., The Salk Institute, La Jolla, California: Ensemble coding of visual information in primate retina.
Dacey, D., University of Washington, Seattle: Parallel visual pathways and retinal microcircuits.
Feller, M., University of California, Berkeley: The mechanisms and function of retinal waves.
Ferster, D., Northwestern University, Evanston, Illinois: Striate cortex 2.
Fitzpatrick, D., Duke University, Durham, North Carolina: The functional architecture of cortical circuits.
Guido, W., Virginia Commonwealth University, Richmond: Development of the retinogeniculate pathway: Structure, function, and underlying mechanisms.
Hirsch, J., University of Southern California, Los Angeles: Comparing thalamus with cortex: Conserved and unique features of neural circuits that build visual receptive fields.
Horton, J., University of California, San Francisco: Striate cortex 1.
Kanwisher, N., Massachusetts Institute of Technology, Cambridge: fMRI investigations of the functional organization of the ventral visual pathway.
Krauzlis, R., The Salk Institute, San Diego, California: Coordination of voluntary eye movements.
Moore, T., Stanford University, Stanford, California: Neural control of visual spatial attention.
Movshon, A., New York University, New York: Visual response properties of MT neurons.
Neitz, J., University of Washington, Seattle: Photopigments and photoreceptors, their evolution, and their role in vision.
Oberdorfer, M., National Institutes of Health, Bethesda, Maryland: NEI/NIH support for vision research.
Ruthazer, E., McGill University, Montreal, Canada: Visual system development in fish and frogs.
Shadlen, M., University of Washington, Seattle: A vision of decision-making.
Sherman, M., University of Chicago, Chicago, Illinois: The role of thalamus in the flow of information to cortex.
Stone, L., NASA Ames Research Center, Moffett Field, California: Visual processing for perception and oculomotor action.
Usrey, M., University of California, Davis: Visual processing in the retinogeniculocortical pathway.

Biology of Memory

July 15–29

INSTRUCTORS
C. Alberini, Mount Sinai School of Medicine, New York
K. Martin, University of California, Los Angeles
J. Raymond, Stanford University, Stanford, California

ASSISTANTS
H. Carlisle, California Institute of Technology, Pasadena
R. Fromke, University of California, San Francisco

This lecture course provided an introduction to cellular, molecular, and systems approaches to learning and memory. It was suited for graduate students and postdoctoral fellows in molecular biology, neurobiology, and psychology as well as research workers who are interested in an introduction to this field. The course covered topics ranging from behavioral considerations of learning and memory to gene regulation in the nervous system. The lectures provided an intensive coverage of modern behavioral studies of learning and memory, the cell and molecular biology of neuronal plasticity, cellular and molecular mechanisms of simple forms of learning and memory, and systems approaches to learning in vertebrates and humans. Lectures were complemented by exercises in which students worked in small groups with lecturers to discuss topical issues in learning and memory, to evaluate recent studies, and to identify and formulate new research questions and approaches. The course was thus designed not only to introduce students to the field of learning and memory, but also to provide an intellectual framework upon which future studies can be built.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Achat-Mendes, C., Ph.D., Harvard Medical School, Southborough, Massachusetts
Arum, O., Ph.D., Southern Illinois University School of Medicine, Springfield
Bambah-Mukku, D., B.S., Mount Sinai School of Medicine, New York
Chen, J., B.S., Stanford University, Stanford, California
Combs-Bachmann, R., B.S., University of Massachusetts, Amherst
De Vito, L., B.S., Boston University, Boston, Massachusetts
Dorrn, A., B.S., University of California, San Francisco
Glerup, S., Ph.D., University of Aarhus, Arhus C, Denmark
Hangya, B., B.S., Institute of Experimental Medicine, Hungary, Academy of Science, Budapest, Hungary
Ho, V., B.S., University of California, Los Angeles
Komorowski, R., B.S., Boston University, Boston, Massachusetts
Li, X., B.S., Columbia University, New York
Manakou, S., B.S., Wellcome Trust Sanger Institute, Cambridge, United Kingdom
McKenzie, S., B.S., Boston University, Boston, Massachusetts
Modi, M., B.S., National Centre for Biological Sciences, Bangalore, India
Netek, A., B.S., University of Texas Medical School, Houston
Pacheco, D., Ph.D., Cayetano Heredia University, Lima, Peru
Phillips, G., B.S., University of California, Irvine
Ramamoorthi, K., B.S., Massachusetts Institute of Technology, Cambridge
Rao, D., B.S., University of North Carolina, Chapel Hill
Xie, X.S., Ph.D., Harvard University, Cambridge, Massachusetts

SEMINARS

Alberini, C., Mount Sinai School of Medicine, New York: Memory consolidation and reconsolidation.
Byrne, J., University of Texas/Houston Medical School, Houston: Aplysia as a model system for studying memory.
Doupe, A. and Brainard, M., University of California, San Francisco: Birdsong and vocal learning.
Eichenbaum, H., Boston University, Boston, Massachusetts: Hippocampal-mediated memories.
Goldman, M., University of California, Davis: Computational approaches to learning and memory.
Holland, P., Johns Hopkins University, Baltimore, Maryland: Learning theory.
Martin, K., University of California, Los Angeles: Cell biology of learning-related plasticity.
Raymond, J., Stanford University, Stanford, California: Cerebellar-mediated memories.
Siegelbaum, S., Columbia University, New York: Synaptic plasticity and information processing in the hippocampus.
Silva, A., University of California, Los Angeles: Molecular cognition; animal models of cognitive disorders.
Squire, L., University of California, San Diego: Historical overview of memory research. Studies of memory in humans.
Waddell, S., University of Massachusetts Medical School, Worchester: *Drosophila* as a model system for studying learning and memory.

Proteomics

July 17–August 1

INSTRUCTORS
T. Andacht, Centers for Disease Control and Prevention, Atlanta, Georgia
J. Bruce, University of Washington, Seattle
A. Link, Vanderbilt University School of Medicine, Nashville, Tennessee
D. Pappin, Cold Spring Harbor Laboratory

ASSISTANTS
A. Farley, Vanderbilt University School of Medicine, Nashville, Tennessee
C. Fu, Cold Spring Harbor Laboratory
P. Kirby, University of Georgia, Athens
C. Ruse, Cold Spring Harbor Laboratory
P. Samir, Vanderbilt University School of Medicine, Nashville, Tennessee
C. Weisbrod, University of Washington, Seattle

This intensive laboratory and lecture course focused on cutting-edge proteomics approaches and technologies. In the protein profiling portion of the course, students gained hands-on experience in several quantitative proteome analysis methods, including two-dimensional gel electrophoresis and isotopic labeling strategies. Students were trained to use DIGE, or differential in-gel electrophoresis, for gel-based protein quantification. Differentially expressed proteins were determined using advanced gel analysis software and identified using MALDI mass spectrometry. Students were

taught differential and quantitative mass spectrometry approaches to profile and identify changes in proteomes. Students were trained in high-sensitivity microcapillary liquid chromatography coupled with nanospray-ESI and tandem mass spectrometry. Both single-dimension and multidimensional microcapillary liquid chromatography separations coupled to mass spectrometry were taught. Students gained hands-on experience purifying and identifying protein complexes and posttranslational modifications using the latest technologies. A strong emphasis was placed on data analysis throughout the course. The overall aim of the course was to provide students with the fundamental knowledge and hands-on experience necessary to be able to perform and analyze proteomics experiments and to learn to identify new opportunities in applying proteomics approaches to their own research.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Bargaje, R., M.S., IGIB, New Delhi, India
Boyer, A., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Chung, J., B.S., UNIFESP, São Paulo, Brazil
Dzijak, R., M.Sc., Institute of Molecular Genetics, Prague, Czech Republic
Evans, T., Ph.D., The University of Iowa, Iowa City
Hao, C., Ph.D., Emory University, Atlanta, Georgia
Islekel, H., Ph.D., Dokuz Eylü University, Izmir, Turkey
Kadam, K., Ph.D., National Institute for Research in Reproductive, Health, Mumbai, India
Klein, I., Dipl., University of Giessen, Germany
Li, W., University of Kentucky, Lexington
Narendra, D., B.A., National Institutes of Health, Bethesda, Maryland
Pattison, S., Ph.D., Queen's University of Belfast, North Ireland
Pratap, S., B.S., Meharry Medical School, Nashville, Tennessee
Savaryn, J., B.S., Medical College of Wisconsin, Milwaukee
Vaitheesvaran, B., Ph.D., SUNY, Stony Brook, New York
Wang, F., Ph.D., Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

SEMINARS

Abbatiello, S., Broad Institute of MIT and Harvard, Cambridge, Massachusetts: MRM and biomarkers.
Arnaudo, A. and Li, T. Princeton University, Princeton, New Jersey: Magnetic isolation of protein complexes.
Chait, B., The Rockefeller University, New York: Proteomic analysis of protein complexes.
Chaurand, P., Vanderbilt University School of Medicine, Nashville, Tennessee: Tissue imaging using mass spectrometry.
Clauser, K., Broad Institute of MIT and Harvard, Cambridge, Massachusetts: De novo interpretation of tandem mass spectra.
Dufresne, C., Thermo Fisher Scientific, West Palm Beach, Florida: Electron transfer dissocation.
Eng, J., University of Washington, Seattle: Tandem mass spectrometry data analysis.
Friedman, D., Vanderbilt University, Nashville, Tennessee: Statistical analysis of two-dimensional DIGE gels.
Kelleher, N., University of Illinois, Urbana: Top-down proteomics.
Peterman, S., Thermo Fisher Scientific, Grimes, Iowa: Analysis of MRM data. Multiple reaction monitoring.
Zhu, H., Johns Hopkins University, Baltimore, Maryland: Design and application of protein microarrays.

Eukaryotic Gene Expression

July 21–August 10

INSTRUCTORS **J. Espinosa,** University of Colorado, Boulder
W.L. Kraus, Cornell University, Ithaca, New York
T. Oelgeschläger, Marie Curie Research Institute, Oxted, United Kingdom
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri

ASSISTANTS **V. Caputo,** Marie Curie Research Institute, Oxted, United Kingdom
R. Henry, University of Colorado, Boulder
C. Lin, Stowers Institute for Medical Research, Kansas City, Missouri
D. Ruhl, Cornell University, Ithaca, New York

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 both on in vitro and in vivo protein–DNA interactions and on novel methodologies to study gene regulation. Students made nuclear extracts, performed in vitro transcription reactions, and measured RNA levels using primer extension. Characterizations of the DNA-binding properties of site-specific transcription factors were carried out using electrophoretic mobility-shift and DNase I footprinting assays. In addition, students learned techniques for the assembly and analysis of chromatin in vitro. These included transcription assays, chromatin footprinting, and chromatin remodeling assays.

During the past few years, the gene regulation field has developed in vivo approaches to study gene regulation. Students were exposed to the chromatin immunoprecipitation technique. They also used RNA interference (RNAi) for specific knockdown experiments in mammalian cells. The

determination of cellular gene expression profiles has been accelerated tremendously by DNA microarray technology, and thus, students received hands-on training in performing and interpreting results from DNA microarrays. Experience with basic recombinant DNA techniques was a prerequisite for admission to this course. Lectures by the instructors covered the current status of the gene expression field, theoretical aspects of the methodology, and broader issues regarding strategies for investigating the regulation of gene expression in eukaryotes. Guest lecturers discussed contemporary problems in eukaryotic gene regulation and technical approaches to their solution.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Alendar, A., Ms.C., Netherlands Cancer Institute, Amsterdam, The Netherlands
Gahura, O., M.S., Charles University, Prague, Czech Republic
Hondele, M., Dipl., EMBL Heidelberg, Germany
Mathew, R., Ph.D., University of Chicago, Illinois
Morgan, M., Ph.D., University of Oxford, United Kingdom
Muramoto, H., M.A., The University of Tokyo, Japan
Pinheiro, A., Licentiate, University of Lisbon, Lisboa, Portugal
Riising, E., M.S., University of Copenhagen, Denmark
Rossmann, M., M.D., Cold Spring Harbor Laboratory
Sankar, S., M.S., University of Utah, Salt Lake City
Sengupta, S., Ph.D., Michigan State University, East Lansing
Shiau, C., B.A., University of California, San Francisco
Tang, C., Ph.D., Cold Spring Harbor Laboratory
Varodayan, F., B.A., Columbia University, New York
Wenzel, S., M.S., University of Bayreuth, Germany
Yang, Y., B.S., University of Helsinki, Finland

SEMINARS

Bartolomei, M., University of Pennsylvania School of Medicine, Philadelphia: Epigenetic regulation of genomic imprinting.
Bernstein, E., Mount Sinai School of Medicine, New York: Epigenetic cross-talk.
Brown, M., Dana-Farber Cancer Institute, Boston, Massachusetts: Mining steroid receptor cistromes.
Dent, S., University of Texas M.D. Anderson Cancer Center, Houston: The importance of a good HAT.
Dermitzakis, M., University of Geneva, Switzerland: Population genetics and genomics of gene expression regulation.
Gingeras, T., Cold Spring Harbor Laboratory: New classes of functional short RNAs and chromosome-wide transcriptional networks.
Kingston, R., Massachusetts General Hospital/Harvard Medical School, Boston: Identification of regulatory mechanisms using high-throughput nucleosome mapping technologies.
Lees, J., Massachusetts Institute of Technology Center for Cancer Research, Cambridge: The RB tumor suppressor–Proliferation, apoptosis, and differentiation regulator.
Levine, M., University of California, Berkeley: Mechanisms of transcriptional precision in the *Drosophila* embryo.
Manley, J., Columbia University, New York: mRNA processing and links to transcription.
Meyer, B., HHMI/University of Berkeley, California: Chromosome-wide control of gene expression.
Nogales, E., HHMI/University of California, Berkeley: Visualizing human protein complexes essential in gene transcription and regulation.
Prives, C., Columbia University, New York: Target gene selection by the p53 tumor suppressor protein.
Pugh, F., Penn State University, University Park, Pennsylvania: Interactions of the transcription regulatory machinery with chromatin on a genomic scale.
Reinberg, D., HHMI/New York University School of Medicine, New York: Transcription and how to be a factor in a crowded field.
Taatjes, D., University of Colorado, Boulder: Molecular mechanisms that regulate mediator coactivator function.
Washburn, M., Stowers Institute for Medical Research, Kansas City, Missouri: Quantitative proteomic analysis of RNA polymerase II and the discovery of a novel Ser-5 CTD phosphatase.

Imaging Structure and Function in the Nervous System

July 21–August 10

INSTRUCTORS **S. Thompson,** University of Maryland School of Medicine, Baltimore
W. Tyler, Arizona State University, Tempe
J. Waters, Northwestern University, Chicago, Illinois

ASSISTANTS **T. Hedick,** Northwestern University, Chicago, Illinois
A. Nimmerjahn, Stanford University, Stanford, California
D. Popovkina, University of Maryland, Baltimore
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 utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser-scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophys-

iological 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. The course used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells.

This course was supported with funds provided by the National Institute of Mental Health, the National Institute of Neurological Disorders & Stroke, and the Howard Hughes Medical Institute.

PARTICIPANTS

Ade, K., Ph.D., Duke University, Durham, North Carolina
Ben Fredj, N., M.Sc., King's College London, United Kingdom
Brunet, I., College of France, Paris
Davie, J., Ph.D., Max-Planck Institute for Medical Research, Heidelberg, Germany
De Biase, L., B.S., Johns Hopkins School of Medicine, Baltimore, Maryland
Direnberger, S., M.S., Max-Planck Institute of Neurobiology, Martinsried, Germany
Elyada, Y., M.A., Max-Planck Institute of Neurobiology, Martinsried, Germany
Huang, A., Ph.D., University of California, San Francisco
Lsasiecka, Z., Ph.D., University of Virginia, Charlottesville
Opazo, P., Ph.D., CNRS–Univerisité de Bordeaux 2, Bordeaux, France
Shepherd, J., Ph.D., Massachusetts Institute of Technology, Cambridge
Thiele, T., Ph.D., University of California, San Francisco

SEMINARS

Bruchez, M., Carnegie Mellon University, Pittsburgh, Pennsylvania: New and future indicators.
Cohen-Cory, S., University of California, Los Angeles: Imaging developmental plasticity.
Deisseroth, K., Stanford University, Stanford, California: Light-triggered activation/silencing.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: Extended 2P imaging and block-face EM.
DiGregorio, D., Université Paris V, Paris, France: Flash photolysis.
Engert, F., Harvard University, Cambridge, Massachusetts: Light sources and detectors. Shot noise. Basics of 2P imaging.
Frostig, R., University of California, Irvine: Intrinsic imaging.
George, N., Olympus America, Inc., Center Valley, Pennsylvania: Objectives. BX51 optics Kohler illumination on commercial.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Bayern, Germany: XFPs and genetically encoded sensors.
Halpain, S., University of California, San Diego, La Jolla: Imaging neuronal morphogenesis. Time lapse and FRAP.
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, Inc., Santa Monica, California: Deconvolution.
Kilborn, K., Intelligent Imaging Innovations, Inc., Santa Monica, California and Redford, G., Intelligent Imaging Innovations, Denver, Colorado: Frequency-domain FLIM.
Lanni, F., Carnegie Mellon University, Pittsburgh, Pennsylvania: Basic microscopy.
Mrsic-Flogel, T., University College London, United Kingdom: Organic calcium indicators in bulk loading. Analysis of Ca bulk loading in vivo.
Murthy, V., Harvard University, Cambridge, Massachusetts: Synaptofluorins and vesicle imaging.
Overstreet-Wadische, L., University of Alabama, Birmingham: Imaging adult-generated neurons.
Smith, S., Stanford Medical School, Stanford, California: Confocal microscopy.
Tanter, M., National Institute for Health and Medical Research, Paris, France: Ultrasound imaging. Analysis.
Tsai, P., University of California, San Diego, La Jolla: Optics bench lab exercises–Basics Koehler. Scanning and fluorescence. Optics bench lab exercises–Scanning. Optics bench lab exercises–Confocal.
Tsai, P., University of California, San Diego, La Jolla and George, N., Olympus America, Inc., Center Valley, Pennsylvania: Optics bench lab exercises–Basics Koehler.
Tyler, J., Arizona State University, Tempe: Image J. Ultrasound imaging.
Waters, J., Northwestern University, Chicago, Illinois: Organic calcium indicators. Single-cell Ca imaging in vivo. Home-brew microscope including laser safety.
Witkowski, J., Cold Spring Harbor Laboratory: Ethics.
Yasuda, R., Duke University Medical Center, Durham, North Carolina: FRET and FLIM.

Yeast Genetics and Genomics

July 21–August 10

INSTRUCTORS **B. Errede,** University of North Carolina, Chapel Hill
J. Vogel, McGill University, Montreal, Canada
M. Whiteway, National Research Council of Canada, Montreal, Canada

ASSISTANTS **E. Elias,** McGill University, Montreal, Canada
E. Nazarova, McGill University, Montreal, Canada
J. Verdassdonk, University of North Carolina, Chapel Hill

This course is a modern, state-of-the-art laboratory course designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches are emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques, including various types of yeast transformation, gene replacement with plasmids and polymerase chain reaction (PCR), construction and analysis of gene fusions, and generation of mutations in cloned genes, were also emphasized. Students used classical and molecular approaches to gain experience in identifying and interpreting various kinds of genetic interactions including suppression and synthetic lethality. They were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluo-

rescence, green fluorescent protein (GFP)–protein fusions and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

This course was supported with funds provided by National Human Genome Research Institute and the Howard Hughes Medical Institute.

PARTICIPANTS

Erbil, W., Ph.D., University of California, Berkeley
Gary, L., Ph.D., GeoSynFuels, Golden, Colorado
Hsu, P.-h., M.S., The Scripps Research Institute, La Jolla, California
Kaur, J., Ph.D., Marquette University, Milwaukee, Wisconsin
Kijanska, M., M.S., ETH Zurich, Switzerland
Kim, S., B.S., University of Oklahoma Health Science Center, Oklahoma City
Kinney, J., Ph.D., Princeton University, Princeton, New Jersey
Li, L., Ph.D., University of Michigan, Ann Arbor
Lilienthal, I., B.S., The Karolinska Institute, Stockholm, Sweden
Milosevic, T., M.S., Mediterranean Institute for Life Sciences, Split, Croatia
Morales, C., B.S., SUNY, Stony Brook, New York
Nagel, Z., Ph.D,. University of California, Berkeley
Qian, W., Ph.D., University of Michigan, Ann Arbor
Ren, X., Ph.D., National Institutes of Health, Bethesda, Maryland
Shieh, G.S., Ph.D., Academia Sinica, Taipei, Taiwan
Wapinski, I., Broad Institute, Cambridge, Massachusetts

SEMINARS

Burke, D., University of Virginia, Charlottesville: Mitotic regulation by checkpoints.
Clarke, D., University of Minnesota, Minneapolis: Mitotic checkpoints and chromosome segregation.
Fink, G., Whitehead Institute for Biomedical Research/MIT, Cambridge, Massachusetts: Evolution of fungal surface proteins and pathogenesis.
Fox, T., Cornell University, Ithaca, New York: Expression of mitochondrial genes in yeast: Localized translation and assembly feedback regulation.
Hughes, T., University of Toronto, Canada: How yeast know where things are in the genome.
Kolfoil, M., McGill University, Montreal, Quebec, Canada: Combining genetic manipulation and sophisticated imaging techniques for studies in yeast.
Lindquist, S., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: The surprising biology of protein conformational change unraveled with yeast.
Luca, F., University of Pennsylvania, Philadelphia: Yeast cell cycle and growth control.
Michnick, S., University of Montreal, Montreal, Canada: Structure and dynamics of protein interaction networks in living cells.
Smith, J., University of Virginia, Charlottesville: The genetics of aging in yeast.
Thorner, J., University of California, Berkeley: From signaling to cell biology: Tales of two scaffolds (Ste5 and septins).
Warner, J., Albert Einstein College of Medicine, Bronx, New York: Yeast and their ribosomes: Key to life, key to growth.
Zakian, V., Princeton University, Princeton, New Jersey: Maintaining the end.

Cellular Biology of Addiction

August 4–10

INSTRUCTORS **C. Evans,** University of California, Los Angeles
M. von Zastrow, University of California, San Francisco

ASSISTANTS **C. Culbertson,** University of California, Los Angeles
V.C. Dang, University of California, San Francisco

Drug addiction is the most costly neuropsychiatric disorder faced by our nation. Acute and repeated exposure to drugs produces neuroadaption and long-term memory of the experience, but the cellular and molecular processes involved are only partially understood. The primary objective of the proposed workshop was to provide an intense dialogue of the fundamentals, state-of-the-art advances, and major gaps in the cell and molecular biology of drug addiction. Targeted to new or experienced investigators, the workshop combined formal presentations and informal discussions to convey the merits and excitement of cellular and molecular approaches to drug addiction research.

With the advent of genomics and proteomics, an extraordinary opportunity now exists to develop comprehensive models of neuroadaptative processes fundamental to addiction, withdrawal, craving, and relapse to drug use and to brain function, in general. A range of disciplines and topics were represented, including noninvasive brain imaging to identify drug targets and adaptive processes; neuroadaptative processes at the molecular and cellular level; neural networks and their modulation; the relevance of genotype to susceptibility and drug response; tolerance and adaptation at the cellular level; and approaches to exploit the daunting volume generated by neuroinformatics. This workshop provided an integrated view of current and novel research on neuroadaptive responses to addiction,

fostered discussion on collaboration and integration, provided critical information needed to construct a model of addiction as a disease and novel molecular targets for biological treatments. Beyond the plane of scientific endeavor, the information is vital for formulating public policy and for enlightening the public on the neurobiological consequences of drug use and addiction. The proposed workshop was designed to generate interest in this level of analysis, open conduits for collaborations, and present novel routes to investigating the neurobiology of addictive drugs.

This course was supported with funds provided by the National Institute of Drug Abuse.

PARTICIPANTS

Angarita, G., Ph.D., Yale University, New Haven, Connecticut
Cazzin, C., Ph.D., Glaxo, Smith & Kline, Verona, Italy
Chen, H., Ph.D., University of Tennessee Health Science Center, Memphis
Davidkova, G., B.S., The Scripps Research Insitute, La Jolla, California
Falcon, E., B.S., University of Texas Southwestern Medical Center, Dallas
Gaskill, P., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Heimer, R., Ph.D., Yale University of Medical School, New Haven, Connecticut
Jin, J., B.S., Pennsylvania State University, Hershey
Khoshbouel, H., Ph.D., Meharry Medical Center, Nashville, Tennessee
Koya, E., Ph.D., National Insitute of Drug Abuse, Baltimore, Maryland
Larson, E., Ph.D., University of Texas Southwestern Medical Center, Dallas
Lugo-Escobar, N., B.S., University of Puerto Rico Medical Science Campus, Rio Piedras
Mohawk, J., Ph.D., University of Virginia, Charlottesville
Reissner, K., Ph.D., Medical University of South Carolina, Charleston
Richards, T., Ph.D., University of Colorado, Denver, Aurora
Salas-Ramirez, K., Ph.D., Sophie Davis School of Biomedical Education Research Center, New York
Scobie, K., B.S., Columbia University, New York
Seabold, G., Ph.D., National Institutes of Health/NIDCD, Bethesda, Maryland
Seasholtz, A., Ph.D., University of Michigan, Ann Arbor
Thompson, R., Ph.D., University of Michigan, Ann Arbor
Tomasiewicz, H., B.S., Mount Sinai School of Medicine, New York
Wood, D., Ph.D., Beth Israel Deaconess Medical School, Boston, Massachusetts

SEMINARS

Augustine, G., Duke University, Durham, North Carolina: Synaptic function and addictive drug action.
Bierut, L., Washington University, St. Louis, Missouri: Human genetics of nicotine dependence.
Blendy, J., University of Pennsylvania, Philadelphia: Functional characterization of gene variants in addiction: Of mice and men.
Bonci, A., University of California, San Francisco: Synaptic plasticity and addiction.
Edwards, R., University of California, San Francisco: Monoamine transporters and their regulation.
Heberlein, U., University of California, San Francisco: Genetic approaches and model organisms in addiction research.
Kalivas, P., Medical University of South Carolina, Columbia: Can we forget to be addicted?
Kieffer, B., Institute of Genetics and Molecular and Cellular Biology, Illkirch, France: Animal models of opioid receptor function and regulation.
Koob, G., Scripps Research Institute, La Jolla, California: Neurocircuitry of addiction: From reward to antireward.
Kreek, M.-J., The Rockefeller University, New York: Endorphins, gene polymorphisms, stress responsivity, and specific addictions: Selected topics.
Mackie, K., University of Indiana, Bloomington: Molecular mechanisms of cannabinoids.
Madras, B., Harvard Medical School, Cambridge, Massachusetts: The public health burden of substance abuse.
Nairn, A., Yale University, New Haven, Connecticut: Dopamine-regulated signal transduction mechanisms and psychostimulant action.
Nestler, E., Mt. Sinai School of Medicine, New York: Molecular mechanisms of drug addiction.
O'Brien, C., University of Pennsylvania, Philadelphia: Translational research in addiction medicine.
Picciotto, M., Yale University, New Haven, Connecticut: Molecular mechanisms underlying nicotine addition.
Pollock, J., National Institute on Drug Abuse/NIH/DHHS, Bethesda, Maryland: NIH/NIDA: Funding and other important resources.
Sulzer, D., Columbia University, New York: Amphetamines and synaptic selection.
von Zastrow, M., University of California, San Francisco and Evans, C., University of California, Los Angeles: Welcome and introduction to addiction as a cell biological problem.
Wightman, M., University of North Carolina, Chapel Hill: Regulation of dopamine release by natural and drug rewards.
Williams, J., Oregon Health & Science Institute, Portland: Dopamine and opioid signaling in single neurons and synaptic connections.

C. elegans

August 14–29

INSTRUCTORS **S. Ahmed,** University of North Carolina, Chapel Hill
E. Miska, University of Cambridge, United Kingdom
M. Zhen, Samuel Lunenfeld Research Institute, Toronto, Canada

ASSISTANTS **A. Clark,** University of Cambridge, United Kingdom
C. Mok, Samuel Lunenfeld Research Institute, Toronto, Canada
M. Simon, University of North Carolina, Chapel Hill

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 investigators who have a current training in molecular biology and some knowledge of genetics, but have no experience with *C. elegans*, and for students with some prior worm experience who wished to expand their repertoire of expertise. The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction and screening of deletion libraries, and RNA inactivation. The course was designed to impart sufficient training to students in the most important attributes of the *C. elegans* system to enable students to embark on their own research projects after returning to their home institutions.

This course was supported with funds provided by the National Institute of Child Health and Human Development.

PARTICIPANTS

Barkoulas, M., Ph.D., University of Oxford, United Kingdom
Castro, C., Ph.D., University of Cambridge, United Kingdom
Chang, A., Ph.D., Duke University, Durham, North Carolina
Dabbish, N., B.S., University of Pennsylvania, Philadelphia
Gleason, R., Ph.D., Rutgers University, Piscataway, New Jersey
Han, L., Ph.D., University of Miami, Florida
Lebois, F., M.S., University Paris Diderot, Paris, France
Levine, E., Ph.D., University of California, La Jolla
Munarriz, E., B.S., New York University, New York
Pruitt, B., Ph.D., Stanford University, Stanford, California
Ramaswamy, P., B.A., University of California, San Francisco
Ramoz, L., B.S., Vanderbilt University, Nashville, Tennessee
Rechavi, O., Ph.D., Tel Aviv University, Israel
Romanowski, A., M.S., Universidad Nacional de Quilmes, Bernal, Argentina
Simon, M., B.S., University of North Carolina, Chapel Hill
Wang, Z., M.D., Duke University, Durham, North Carolina
Wen, Q., Ph.D., Janelia Farm Research Campus, HHMI, Ashburn, Virginia

SEMINARS

Ahmed, S., University of North Carolina, Chapel Hill: Telomeres and telomerase.
Ahringer, J., University of Cambridge, United Kingdom: Cell polarity and chomatin.
Alkema, M., University of Massachusetts Medical School, Cambridge: Behavioral genetics.
Blumenthal, T., University of Colorado, Boulder: RNA processing.
Culotti, J., Samuel Lunenfeld Research Institute, Toronto, Canada: Axonal pathfinding.
Fang-Yen, C., Harvard University, Cambridge, Massachusetts: Calcium imaging in *C. elegans*.
Felix, M.-A., Institut Jacob Monod, CNRS, Paris, France: *C. elegans* evolution.
Hall, D., Albert Einstein College of Medicine, Bronx, New York: Ultrastructural analysis.
Kim, D., Massachusetts Institute of Technology, Cambridge: Pathogenesis.
Miska, E., University of Cambridge, United Kingdom: microRNAs.
Moerman, D., University of British Columbia, Vancouver, Canada: Toward a mutation in every gene: The knockout facility and application of new technologies.
Murphy, C., Princeton University, Princeton, New Jersey: Mechanisms of aging.
Piano, F., New York University, New York: Genomics and systems biology approaches in *C. elegans*.
Richmond, J., University of Illinois, Chicago: Pre- and post-synaptic regulation of *C. elegans* synaptic transmission.
Zhen, M., Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Canada: Nervous system development.

Programming for Biology

October 12–27

INSTRUCTORS **S. Prochnik,** DOE–Joint Genome Institute/University of California, Berkeley
J. Tisdall, DuPont Experimental Station, Wilmington, Delaware

ASSISTANTS **J. Aerts,** Roslin Institute, Scotland, United Kingdom
J. Babayev, Pioneer Hi-Bred, Johnston, Iowa
E. Lee, Lawrence Berkeley National Laboratory, Berkeley, California
J. Leipzig, DuPont Experimental Station, Wilmington, Delaware
D. Messina, Washington University School of Medicine, St. Louis, Missouri
S. Robb, University of Utah, Salt Lake City

The 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 became even more important as new technologies increased the already exponential rate at which biological data are 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 this 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 each other and the faculty to solve them. Final projects formed the basis of publications as well as public biological Web sites (see, for example, http://bio.perl.org/wiki/Deobfuscator).

The prerequisite for the course was a 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. Note that 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.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

An, Y.-q., Ph.D., Agricultural Research Service, United States Department of Agriculture, St. Louis, Missouri
Ayroles, J., Ph.D., North Carolina State University, Raleigh
Blackman, B., Ph.D., Duke University, Durham, North Carolina
Bonin, A., Ph.D., Indiana University, Bloomington
Brawley, S., Ph.D., University of Maine, Orono
Burki, F., Ph.D., University of British Columbia, Vancouver, Canada
Coruh, C., Ph.D., Pennsylvania State University, State College
Gregg, C., Ph.D., Harvard University, Cambridge, Massachusetts
Joneson, S., Ph.D., Duke University, Durham, North Carolina
Kolokotronis, S.-O., B.S., American Museum of Natural History, New York
Matas Arroyo, A.J., B.S., Cornell University, Ithaca, New York
Mount, S., Ph.D., University of Maryland, College Park
Nordlund, J., B.S., Uppsala University, Sweden
Pezer, Z., M.Sc., Ruder Boskovic Institute, Zagreb, Croatia
Reis E., Ph.D., University of São Paulo, Brazil
Ronan, J., B.A., Stanford University, Stanford, California
Serebriiskii, I., Ph.D., The Fox Chase Cancer Center, Philadelphia, Pennsylvania
Siena, E., M.S., University of Perugia, Italy
Sierra, R., M.S., Universidad de los Andes, Bogota, Colombia
Simon, S., M.S., Stiftung Tieraerztliche Hochschule Hannover, Germany
Slot, J., Ph.D., Vanderbilt University, Nashville, Tennessee
Wolf, A., A.B., Harvard University, Boston, Massachusetts

SEMINARS

Begovic, E., University of California, Berkeley: Molecular evolution and population genetics. Molecular evolution and phylogenetics.
Cain, S., Ontario Institute for Cancer Research, Medina, Ohio: Gbrowse.
Deschamps, S., Dupont, Wilmington, Delaware: NextGen sequencing 1.
Dos Santos, S., Smithsonian Tropical Research Institute, Panama City, Panama: Bioinformatics in tropical field research sequencing samples.
Hide, W., Harvard University/SANBI, Boston, Massachusetts: EST assembly and analysis.
Lee, E., University of California, Berkeley: Perl III–Arrays and hashes.
Leipzig, J., Dupont, Philadelphia, Pennsylvania: HTML. NextGen sequence assembly III.
McKay, S., Cold Spring Harbor Laboratory: Database design. DBI. CGI.
Messina, D., Stockholm University, Sweded: Perl VI–Subroutines and modules.
Pearson, W., University of Virginia, Charlottesville: Sequence similarity searches and blast I. Sequence similarity searches and blast II.
Prochnik, S., DOE Joint Genome Institute, Walnut Creek, California: Perl IV–References. Perl VII–Object-oriented Perl. Perl pipelines.
Robb, S., University of Utah, Salt Lake City: Bioperl I. Bioperl II. Using UNIX. Perl II–Conditionals, logicals operators, loops, and filehandles. Perl V–Regular expressions.
Thomas, P., SRI International, Menlo Park, California: Predicting protein function.
Tisdall, J., DuPont Experimental Station, Wilmington, Delaware: Scientific computing. Perl I–Program structure, statements, variables.
Vaugh, M., Cold Spring Harbor Laboratory: NextGen sequencing II.

X-ray Methods in Structural Biology

October 12–27

INSTRUCTORS **W. Furey,** V.A. Medical Center/University of Pittsburgh, Pittsburgh, Pennsylvania
G. Gilliland, Centorcor R&D, Inc., Radnor, Pennsylvania
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku Americas Corporation, The Woodlands, Texas

ASSISTANT **R. Kettering,** University of Pittsburgh, Pittsburgh, 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 to be covered include 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 the theory and informal discussions behind the techniques. Applicants should be familiar with the creation and editing of simple text files on Linux workstations using a screen based editor (either vi or emacs).

This course was supported with funds provided by the National Cancer Institute and the Howard Hughes Medical Institute.

PARTICIPANTS

Baconguis, I., B.A., Oregon Health and Science University, Portland
Balcewich, M., B.Sc., University of Manitoba, Winnipeg, Canada
Diver, M., B.S., Memorial Sloan-Kettering Cancer Center, New York
Fuentes, E., Ph.D., University of Iowa, Iowa City
Guglielmi, K., Ph.D., National Institutes of Health, Bethesda, Maryland
Hauk, G., B.S., Johns Hopkins University, Baltimore, Maryland
Johnson, T., B.S., Kansas University Medical Center, Kansas City
Menichelli, E., Laureate, The Scripps Research Institute, La Jolla, California
Page, R., Ph.D., Cleveland Clinic Foundation, Cleveland, Ohio
Prince, D., M.S., AstraZeneca, Waltham, Massachusetts
Puglisi, J., Ph.D., Stanford University School of Medicine, Stanford, California
Shaw, P.-C., Ph.D., The Chinese University of Hong Kong, China
Takagi, Y., Ph.D., Indiana University, Indianapolis
Tonthat, N., B.S., M.D. Anderson Cancer Center, Houston, Texas
VanOs, L., B.S., University of Oregon, Eugene
Washington, T., Ph.D., University of Iowa College of Medicine, Iowa City

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, Berkeley, California: Introduction to PHENIX (and CNS). Structure refinement.
Cohen, A., Stanford Synchrotron Radiation Laboratory, Stanford, California: Remote synchrotron data collection.
Emsley, P., University of York, United Kingdom: Model-building tools in coot.
Furey, W., V.A. Medical Center/University of Pittsburgh, Pittsburgh, Pennsylvania: Anomalous data collection consideration. Patterson group therapy. Isomorphous replacement and anomalous scattering. Solvent flattening/phase combination. Noncrystallographic symmetry averaging. MAD Phasing: A classical approach. Solving structures with BnP.
Gilliland, G., Centorcor Inc., Radnor, Pennsylvania: Course overview: The structure determination of biological macromolecules. Maximizing crystallization success through seeding. Review/discussion and fill out evaluations.
Joshua-Tor, L., Cold Spring Harbor Laboratory: DNA translocation in a replicative hexameric helicase.
Kleywegt, G., EMBL-EBI, Cambridge, United Kingdom: Just because it's in Nature, doesn't mean it's true… .
Kumar, P.R., Cold Spring Harbor Laboratory: Pymol animation.
McPherson, A., University of California, Irvine: Crystallization of macromolecules I. Crystallization of macromolecules II. Symmetry, periodicity, unit cells, space groups, Miller planes, and lattices. Waves, vectors, and complex numbers. Fundamental diffraction relationships and Bragg's law. Diffraction patterns, reciprocal space and Ewald's sphere. Fourier transforms and the electron density equation. Patterson methods. Crystallization review. Heavy atoms and anomalous scatterers. Mechanisms of crystal growth.
Perrakis, A., Netherlands Cancer Institute, Amsterdam, The Netherlands: Automated model building and refinement with ARP/WARP.
Pflugrath, J., Rigaku Americas Corporation, The Woodlands, Texas: Data dollection: Design and setup. Cryocrystallography. d*Trek/HKL2000 tutorial. Scaling and merging synchroton data.
Read, R., University of Cambridge, United Kingdom: Molecular replacement: New structures from old. Using SAD data in phaser.
Richardson, J., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using MolProbity.
Sweet, R., Brookhaven National Laboratory, Upton, New York: Fundamentals of crystallography. X-ray sources and optics.
Terwilliger, T., Los Alamos National Laboratory, Los Alamos, New Mexico: Automated structure solution and model building.
Tronrud, D., Oregon State University, Corvallis: Macromolecular refinement I. Macromolecular refinement II. Difference electron density maps.
Westbrook, J., Rutgers, The State University of New Jersey, Piscataway: Automating PDB deposition.
Yeh, J., University of Pittsburgh, Pittsburgh, Pennsylvania: Approaches for membrane protein crystallization. Assessing crystallizability via biophysical characterization.

Workshop on Cereal Genomics

October 13–19

INSTRUCTORS
S. Hake, Plant Gene Expression Center, Albany, California
D. Jackson, Cold Spring Harbor Laboratory
D. Ware, Cold Spring Harbor Laboratory/USDA-ARS

ASSISTANTS
A. Eveland, Cold Spring Harbor Laboratory
D. O'Connor, University of California, Berkeley
S. Pasternak, Cold Spring Harbor Laboratory
P. Van Buren, Cold Spring Harbor Laboratory

This 1-week workshop enabled participants to take advantage of emerging genetic tools and the completed cereal genome sequences of rice and maize, and emerging sequences of other species. The workshop featured morning and evening lectures with afternoon computer lab exercises. It also provided hands-on lab work in comparative anatomy, phenotype, and QTL sections in which participants examined samples and made measurements for statistical analysis to illustrate the power of maize genetics and its relation to genomics. The faculty (instructors and invited lecturers) are active researchers in cereal genetics and genomics who have made significant contributions to the field, ensuring that the latest techniques and ideas were presented. The course was structured to provide time for informal discussions and exchange with leaders in the field.

This workshop was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Aquino, G., M.S., International Rice Research Institute, Metro Manila, Philippines
Cruz, G., B.S., University of São Paulo, Brazil
DeRose-Wilson, L., Ph.D., Colorado State University/University of California, Irvine, Fort Collins
Dorweiler, J., Ph.D., Marquette University, Milwaukee, Wisconsin
Gasperini, D., M.Sc., John Innes Centre, Norwich, United Kingdom
Guo, X., B.S., University of Nebraska, Lincoln
Gutierrez-Nava, L., Ph.D., DuPont, Wilmington, Delaware
Gutijahr, C., Ph.D., University of Lausanne, Switzerland
Herath, V., B.Sc., University of Maine, Orono
Hill, D., B.S., University of Wyoming, Laramie
Madzima, T., Ph.D., Florida State University, Tallahassee
Oono, Y., Ph.D., National Institute of Agrobiological Sciences, Tsukuba, Japan
Petsch, K., Ph.D., Cold Spring Harbor Laboratory
Rijavec, T., Ph.D., National Institute of Biology, Ljubljana, Slovenia
Santa Cruz Hidalgo, J., B.S., North Carolina State University, Raleigh
Sosso, Davide, M.S., Ecole Normale Superieure de Lyon, France

SEMINARS

Bennetzen, J., University of Georgia, Athens: Orphan cereal genomics: Comparative power and the search for novelty.
Buckler, E., Cornell University, New York and Rocheford, T., Purdue University, Indiana: QTL analyses.
Devos, K., University of Georgia, Athens: Genomics in nonmodel grass species: Challenges and strategies.
Harper, L., Maize GDB/University of Berkeley, California: Genomes, chromosomes, genes, and how maize GDB and other cereal databases can help you.
Kellogg, E., University of Missouri, St. Louis: Diversity of the cereals and other grasses.
Koch, K., University of Florida, Gainesville: Transposons for reverse genetics in maize.
Meyers, B., University of Delaware, Newark: New technologies for RNA analysis.
Rafalski, A., DuPont/Pioneer, Wilmington, Delaware: Association analyses.
Vielle-Calzada, J.P., National Laboratory of Genomics for Biodiversity, Irapuato, Mexico: Genomics and maize land races.
Wessler, S., University of Georgia, Athens: How transposable elements amplify throughout genomes.

Advanced Sequencing Technologies and Applications

November 3–16

INSTRUCTORS

E. Mardis, Washington University School of Medicine, St. Louis, Missouri
G. Marth, Boston College, Chestnut Hill, Massachusetts
W. McCombie, Cold Spring Harbor Laboratory
J. McPherson, Ontario Institute for Cancer Research, Toronto, Canada
M. Zody, Broad Institute, Cambridge, Massachusetts

ASSISTANTS

D. Barnett, Boston College, Chestnut Hill, Massachusetts
M. Busby, Boston College, Chestnut Hill, Massachusetts
L. Cardone, Cold Spring Harbor Laboratory
Y. Erlich, Cold Spring Harbor Laboratory
E. Hodges, Cold Spring Harbor Laboratory
A. Indap, Boston College, Chestnut Hill, Massachusetts
M. Kramer, Cold Spring Harbor Laboratory
V. Magrini, Washington University School of Medicine, St. Louis, Missouri
S. McGrath, Washington University School of Medicine, St. Louis, Missouri
N. Navin, Cold Spring Harbor Laboratory
J. Parla, Cold Spring Harbor Laboratory
A. Quinlan, University of Virginia, Charlottesville
C. Stewart, Boston College, Chestnut Hill, Massachusetts
C. Teiling, Roche Diagnostics/454, Indianapolis, Indiana
J. Yao, Cold Spring Harbor Laboratory

During the last decade, large-scale DNA sequencing has markedly impacted the practice of modern biology and is beginning to affect the practice of medicine. With the recent introduction of several advanced sequencing technologies, costs and timelines 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 2-week 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 was 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.

PARTICIPANTS

Bebek, G., Ph.D., Case Western Reserve University, Cleveland, Ohio
Clark, L., Ph.D., Columbia University, New York
Cohen, H., Ph.D., Bar-IIan University, Ramat Gan, Israel
Cox, M., Ph.D., Massey University, Palmerston North, New Zealand
Doktor, T., M.S., University of Southern Denmark, Odense, Denmark
Elferink, M., M.S., Wageningen University, The Netherlands
Grosso, A., Ph.D., Institute of Molecular Medicine, Lisboa, Portugal
Hon, C.C., Ph.D., Institut Pasteur, Paris, France
Kao-Kniffin, J., Ph.D., University of Wisconsin, Madison
Konopka, G., Ph.D., University of California, Los Angeles
Kwon, S.Y., Ph.D., University of Birmingham, Edgbaston, United Kingdom
Paisan-Ruiz, C., Ph.D., University College London Institute of Neurology, London, United Kingdom
Pluvinet, R., Ph.D., IMPPC, Barcelona, Spain
Schlegel, S., Dipl., Stockholm University, Stockholm, Sweden
Simoes, B., Lic., University College Dublin, Republic of Ireland
Velichutina, I., Albert Einstein College of Medicine, Bronx, New York

SEMINARS

Angly, F., San Diego State University, San Diego, California: Metagenomics.
Brayer, J., RainDance Technologies, Inc., Lexington, Massachusetts: Targeted enrichment by emulsion PCR and the RainDance RDT 1000.
Dooling, D., The Genome Center at Washington University, St. Louis, Missouri: IT infrastructure for NGS.
Eichler, E., The University of Washington, Seattle: Human genome structural variation discovery by next-generation sequencing.
Gnirke, A., Broad Institute, Cambridge, Massachusetts: Solution-phase capture of DNA or RNA with SureSelect.
Maher, C., University of Michigan, Ann Arbor: Discovery of fusion transcripts in RNA-Seq data.
Mardis, E., The Genome Center at Washington University, St. Louis, Missouri: Whole-genome sequencing, variant detection, and structural variation in cancer genomes.
McPherson, J., The Ontario Institute for Cancer Research, Toronto, Canada: Next-generation sequencing of cancer genomes at the OICR.
Mikkelson, T., Broad Institute, Cambridge, Massachusetts: Comparative epigenomics of human and murine adipogenesis.
Overduin, B., EBI-European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom: Short-read archive.
Russ, C., Broad Institute, Cambridge, Massachusetts: Helicos sequencer.
Schroth, G., Illumina, Inc., Hayward, California: RNA-Seq on the Illumina GAIIx.
Stuart, J., Life Technologies, Inc., Carlsbad, California: cDNA sequencing by SOLiD.
Turner, S., Pacific Biosciences, Menlo Park, California: Real-time single-molecule (SMRT) sequencing.
Watson, J., Cold Spring Harbor Laboratory: My genome sequencing.

Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

November 3–16

INSTRUCTORS **V. Allan,** University of Manchester, United Kingdom
K. Hu, Indiana University, Bloomington
J. Murray, University of Pennsylvania School of Medicine, Philadelphia

COINSTRUCTOR **J. Peng,** Stanford University, Stanford, California

ASSISTANTS **A. Heaslip,** University of Indiana, Bloomington
P. March, University of Manchester, United Kingdom
A. McEvoy, University of California, Berkeley
Y.-C. Wang, University of California, Riverside

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, and several superresolution methods including structured illumination, STORM and PALM digital image processing, and time-lapse imaging of living specimens. It was designed to present students with state-of-the-art technology and scientific expertise in the use of light microscopy to address basic questions in cellular and molecular biology. The course was designed for

the molecular biologist who is in need of microscopic approaches and for the cell biologist who is not familiar with the practical application of the advanced techniques presented in the course. Among the methods presented were the preparation of tagged nucleic acid probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, comparative genomic hybridization, cellular localization of RNA, localization of nucleic acids and proteins in the same cells, use of a variety of reporter molecules and nonantibody fluorescent tags, indirect antibody labeling, detection of multiple proteins in a single cell labeling antibodies with two fluorophores for STORM, and the use of green fluorescent protein (GFP) variants to study protein expression, by wide field on PALM, localization, and dynamics. In each method, several experimental protocols were presented allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring their own nucleic acid, protein, or antibody probes to the course, which were used in addition to those provided by the instructors. The laboratory exercises were supplemented with lectures given by invited distinguished scientists, who presented up-to-the-minute reports on current methods and research using the techniques being presented.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Barry, J., B.A., Columbia University, New York
Bergmann, J., Dipl., The University of Edinburgh, United Kingdom
Campbell, E., Ph.D., Loyola University, Maywood, Illinois
Chen E.-S., Ph.D., National University Singapore, Singapore
Duquette, A., Ph.D., McGill University, Montreal, Canada
Estévez-Salmerón, L., Ph.D., University of California, San Francisco
Keim, C., B.S., Columbia University, New York
Konings, I., B.A., Wageningen University and Research Centre, The Netherlands
Lauks, J., M.Sc., Center for Neurogenomics & Cognitive Research, Amsterdam, The Netherlands
Mesentier-Lourc, L., B.A., UFRJ, Rio de Janeiro, Brazil
Oguro, A., Ph.D., University of California, Los Angeles
Paul, S., B.S., Uniformed Services University of the Health Sciences, Bethesda, Maryland
Perez, O., Ph.D., SDB-LASDB, Quito, Ecuador
Schosserer, M., M.Sc., University of Applied Life Sciences, Vienna, Austria
Siddiqui, T., Ph.D., University of British Columbia, Vancouver, Canada
von der Chevallerie, K., M.S., Tierärztliche Hochschule Hannover, Germany

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.
Goodwin, P., Applied Precision, Inc., Issaquah, Washington: Introduction to structured illumination methods.
Hu, K., Indiana University, Bloomington and Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Introduction to light and fluorescence microscopy.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Digital detectors and digital imaging fundamentals.
North, A., The Rockefeller University, New York: Immunocytochemistry on tissue sections: Principles and practice.
Ried, T., National Cancer Institute/NIH, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Shroff, H., National Institutes of Health, Bethesda, Maryland and Bates, M., Harvard University, Cambridge, Massachusetts: Pointlist imaging: PALM, STORM, and similar methods.
Spector, D., Cold Spring Harbor Laboratory: Visualizing gene expression/silencing in living cells.

Computational and Comparative Genomics

November 4–10

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
R. Smith, GlaxoSmithKline, King of Prussia, Pennsylvania
L. Stubbs, University of Illinois, Urbana

ASSISTANT **D. Triant,** Louisana 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. The course focused on approaches for extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, and multiple sequence alignment. Additional topics included gene recognition (exon/intron prediction), identifying signals in unaligned sequences, and integration of genetic and sequence information in biological databases. The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course made extensive use of local WWW pages to present problem sets and the computing tools to solve them. Students used Windows and Mac workstations attached to a UNIX server; participants 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 for 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.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Barski, A., Ph.D., National Heart, Lung and Blood Institute/ NIH, Bethesda, Maryland
Bratcher, H., M.Sc., University of Oxford, United Kingdom
Buza, T., Ph.D., Mississippi State University, Mississippi State
Crowgey, E., M.S., Pioneer Hi-Bred, Wilmington, Delaware
Doucet, J., Ph.D., Nicholls State University, Thiboaux, Louisiana
Gallach, M., M.S., University of Texas, Arlington
Harrison, O., Ph.D., Oxford University, United Kingdom
Hnath, J., M.S., National Bioforensic Analysis Center, Frederick, Maryland
Kabara, E., B.S., Michigan State University, East Lansing
Kamir, D., M.Sc., The Jackson Laboratory, Bar Harbor, Maine
Kienle, N., M.S., Max-Planck Institute for Biophysical Chemistry, Goettingen, Germany
Lewis, S., B.S., University of California, Riverside
Lin, Y., M.D., RIKEN, Kobe, Japan
Lopes, A., B.Sc., IPATIMUP, Porto, Portugal
Low, L., Ph.D., Malaysian Palm Oil Board, Bandar Baru, Bangi, Malaysia
Matthiesen, R., Ph.D., IPATIMUP, Porto, Portugal
Muhammad, A., D.Sc., Tuskegee University, Tuskegee, Alabama
Schwarzbach, A., Ph.D., University of Texas, Brownsville
Seiler, M., Ph.D., University of Chicago, Chicago, Illinois
Stanton, J.-A., Ph.D., University of Otago, Dunedin, New Zealand
Zweifel, E., B.S., St. Olaf College, Northfield, Minnesota

SEMINARS

Altschul, S., National Library of Medicine, Bethesda, Maryland: Statistics of sequence similarity scores. Iterated protein database searches with PSI-BLAST.
Cooper, P., National Center for Biotechnology Information/National Library of Medicine, Bethesda, Maryland: NCBI resources for bioinformatics and computational biology. MCBO genome resources.
Overduin, B., EMBL–European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom: The ENSEMBL database of genomes I: ENSEMBL II. Compara.
Pearson, W., University of Virginia, Charlottesville: Protein evolution and sequence similarity searching. Similarity searching II–strategies. Suboptimal global alignments. Hidden Markov models and proteins profiles. Alignment algorithms–Large-scale alignment I. Consensus sites.
Smith, R., GlaxoSmithKline, King of Prussia, Pennsylvania: Approaches to multiple sequence alignment/multiple alignment resources. Multiple alignment resources.
Stubbs, L., University of Illinois, Urbana: Genome comparison biology.
Thomas, P., SRI International, Menlo Park, California: Interfering function/orthology.

Phase Display of Proteins and Peptides

November 9–16

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
L. Yunk, University of Pennsylvania, Philadelphia

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This intensive laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. ScFu antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *Escherichia coli* were also covered. Antibody epitopes were selected from peptide libraries and characterized. The lecture series, presented by a number of invited speakers, emphasized polymerase chain reaction (PCR) of immunoglobulin genes, the biology of filamentous phage, and the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, directed protein evolution, cell display libraries,

the immunobiology of the antibody response, and recent results on the use of antibodies in therapy. The theory and practical implications for selection from phage displayed libraries of random peptides, cDNA products, and semisynthetic proteins were also explored.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Alexis, F., Ph.D., Clemson University, Clemson, South Carolina
Andersson, O., M.S., Karolinska Institutet, Stockholm, Sweden
Dunn, R., B.A., Biogen Idec, San Diego, California
Felizzola, O., B.S., Institution of Advanced Studies, Caracas, Venezuela
Gadermaier, E., M.Sc., Center for Physiology and Pathophysiology, Vienna, Austria
Jung, J., Ph.D., Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea
Madala, S., M.Sc., National Institutes of Health/NIAID, Bethesda, Maryland
Malm, M., M.Sc., Royal Institute of Technology, Stockholm, Sweden
Martinez-Barnetche, J., B.S., Instituto Nacional De Salud Publica, Cuernavaca, Mexico
Matsuo, A., Ph.D., Universidade Federal de São Paulo, Brazil
Moreland, N., Ph.D., Duke NUS Graduate Medical School, Singapore
Nilsson, O., M.Sc., Karolinska Institutet, Stockholm, Sweden
Ong, Y.T., B.S., University of Missouri, Columbia
Puffer, B., Ph.D., Integral Molecular, Philadelphia, Pennsylvania
Stambolsky, P., Ph.D., Weizmann Institute of Science, Rehovot, Israel
Trilling, A.K., M.S., Plant Research International, Wageningen, The Netherlands

SEMINARS

Almagro, J.C., Johnson & Johnson, Radnor, Pennsylvania: Antibody humanization.
Barbas, C., The Scripps Research Institute, La Jolla, California: Software and hardware for genomes: Polydactyl zinc finger proteins and the control of endogenous genes.
Noren, C., New England Biolabs, Beverly, Massachusetts: Phage peptide libraries: The Ph.D. for peptides.
Rader, C., National Cancer Institute/NIH, Bethesda, Maryland: Monoclonal antibody drug and target discovery for cancer therapy.
Sidhu, S., Genetech, Inc., S. San Francisco, California: Antibody phage display and chemical diversity in antigen recognition.
Siegel, D., University of Pennsylvania Medical Center, Philadelphia: Cell surface selection of combinatorial Fab libraries.
Silverman, G., University of California, San Diego, La Jolla: Repertoire cloning of SLE autoantibodies.
Smith, G., University of Missouri, Columbia: Phage display of peptides.
Stanfield, R., The Scripps Research Institute, La Jolla, California: Structural biology of the immune system.
Wittrup, K.D., Massachusetts Institute of Technology, Cambridge: Yeast display libraries.

The Genome Access Course

April 20–21, November 17–18

TRAINERS **G. Howell,** The Jackson Laboratory
B. King, The Jackson Laboratory
L. Reinholdt, The Jackson Laboratory
R. Sachidanandam, Cold Spring Harbor Laboratory

This course was an intensive 2-day introduction to bioinformatics that was held twice in 2009 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 consisting of a discussion of theory and methods, coverage of software and web resources, and use of selected tools with examples (including those supplied by the students). The modular design allowed the instructors to tailor the presentation to the interests of the students. Modules included Electronic Sequence Information; Pairwise Sequence Comparisons; Multiple Sequence Alignments; Gene Prediction; Genome Analysis; Sequence Variation; Protein Classification and Structural Analysis; Proteomics; and Phylogenetic Analysis. Applications to the course were open to all on a first-come–first-served basis, subject to basic eligibility requirements. The course was held at the Laboratory's Genome Research Center at Woodbury located seven miles south of the main Laboratory campus. Each student was provided with a PC laptop with wireless modem for the duration of the course. Students were encouraged to supply problem sets and sequences of interest to the trainers for possible incorporation as examples in the modules. Materials were made available on the web and students continued to ask questions of the trainers as they applied what they had learned in their individual endeavors.

April 20–21

Alonso, A., Mount Sinai School of Medicine, Genetics and Genomic Sciences, New York
Backman, C., NIH/NIDA Intramural Research Program, Cellular Neurobiology Research Branch, Baltimore, Maryland
Basso, M., Burke-Cornell Medical Research Institute, Neurology and Neurosciences, White Plains, New York
Belmonte Mahon, P., Johns Hopkins University, Psychiatry, Baltimore, Maryland
Chandra, D., Roswell Park Cancer Institute, Pharmacology and Therapeutics, Buffalo, New York
DiLaura, R., Cleveland Clinic and Case Western Reserve University, Clinical and Translational Research Informatics, Brecksville, Ohio
Forslund, A., Albert Einstein College of Medicine, Genetics, Bronx, New York
Gravina, S., Albert Einstein College of Medicine, Genetics, Bronx, New York
Jensen, M., Harvard School of Public Health, Nutrition/Epidemiology, Boston, Massachusetts
Ma, T., Burke Medical Research Institute, Research–Neurology and Neuroscience, White Plains, New York
Majovski, R., *Genome Research*, Cold Spring Harbor Laboratory
Mayeux, R., Columbia University College of Physicians & Surgeons, Taub Institute, New York
Parla, J., Cold Spring Harbor Laboratory, Genome Research Center, Woodbury, New York
Qin, W., James J. Peters VA Medical Center, RR&D Center of Excellence, Bronx, New York
Rogers, J., Baylor College of Medicine, Human Genome Sequencing Center, Houston, Texas
Stowe, A., Washington University in St. Louis, Neurosurgery, St. Louis, Missouri
Willour, V., Johns Hopkins University, Psychiatry and Behavioral Sciences, Baltimore, Maryland
Wray, C., MDIBL, Education, Salisbury Cove Maine

November 15–18

Chellani, M., Translational Research, Melville, New York
Choi, G., University of Chicago, Surgery, Chicago, Illinois
Glaser, R., Stevenson University, Biology, Stevenson, Maryland
Hartung, A., GNF, Oncology Drug Discovery, San Diego, California
Imumorin, I., Cornell University, Department of Animal Science, Ithaca, New York
Lau, F., Harvard Stem Cell Institute, Cowan Lab, Boston, Massachusetts
Lenert, G., University of Iowa Library, Hardin Library for the Health Sciences, Iowa City
Levin, M., University of Pennsylvania, Pediatrics, Division of Cardiology, Philadelphia
Lin, C.-Y., Albert Einstein College of Medicine, Epidemiology and Public Health, Bronx, New York
MacKinnon, C., University of the Incarnate Word, Biology, San Antonio, Texas
Meiss, S., California University of Pennsylvania, Biological and Environmental Sciences, California, Pennsylvania
Mendoza, O., Stella Maris College (Autonomous), Bioinformatics, Chennai, India
Owens, E., University of Alabama, Microbiology, Birmingham
Parker, J., Columbia University, Genetics and Development, New York
Paul, J., DuPont, CR&D, Wilmington, Delaware
Rosner, M., University of Chicago, Ben May Department for Cancer Research, Chicago, Illinois
Sellors, J., United Kingdom Biobank, Operations/Legal, London, United Kingdom
Sun, S., Cold Spring Harbor Laboratory
Taylor, K., University of Missouri, Pathology and Anatomical Sciences, Columbia
Tran, S., Amgen, Inc., Investigative Toxicology, Thousand Oaks, California
Zhang, W., Roswell Park Cancer Institute, Molecular Pharmacology and Cancer Therapeutics, Buffalo, New York

Cold Spring Harbor Laboratory acknowledges the generosity of the following companies that loaned equipment and reagents to the various courses:

454 Life Sciences
Accuri Cytometers Inc.
Affymetrix
Agilent Technologies Inc.
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Applied Biosystems
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Bruker Daltonics Inc.
Carl Zeiss Inc.
Charles River Laboratories
Chroma Technology Corporation
Coherent Laser
ConOptics
Corning Inc.
CrystaLaser
David Kopf Instruments
Doric Lenses
Drummond Scientific Company
Epicentre Technologies
Eppendorf North America
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Varian Inc., Consumable Products
VDS Vosskuhler GmbH
Warner Instruments
Waters Corporation
World Precision Instruments
Zeitz-Instrumente Vertriebs GmbH

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. Jeff Lichtman, Harvard University	Connectomics in the developing nervous system.	Josh Huang
Dr. Li-Heui Tsai, Massachusetts Institute of Technology	Chromatin remodeling in neurodegeneration and neuronal repair.	Josh Huang
Dr. Titia de Lange, The Rockefeller University	How telomeres protect chromosome ends.	David Spector
Dr. Lisa Miller, Brookhaven National Laboratory	Ex situ imaging of amyloid structure and metal binding in a mouse model of Alzheimer's disease.	Leemor Joshua-Tor
February		
Dr. Michael Ehlers, Duke University	Protein machinery for postsynaptic plasticity.	Hiro Furukawa
Dr. Robin Lovell-Badge, National Institute for Medical Research, London	How the decision to be male or female is made and maintained in mammals.	Leemor Joshua-Tor
Dr. Caroline Dean, John Innes Centre, United Kingdom	Chromatin silencing in flowering time control.	Rob Martienssen
March		
Dr. David Glover, University of Cambridge	Organizing centrosomes and assembling centrioles: Roles of the polo-like kinases 1 and 4.	David Spector
Dr. Ronald DePinho, Harvard University/ Dana Farber Cancer Institute	FoxOs in cancer and aging.	Terri Grodzicker
Dr. David Porteous, The University of Edinburgh	Genetics and biology of psychosis: Learning from DISC1.	Dick McCombie
Dr. Robert Desimone, Massachusetts Institute of Technology	Neural synchrony and selective attention.	Tony Zador
April		
Dr. John Kuriyan, University of California, Berkeley	Allosteric mechanisms in the activation of the EGF receptor.	Bruce Stillman
Dr. Lewis Cantley, Harvard University	PI3 kinase, cell growth regulation, and disease.	Scott Powers
Dr. Thomas Sudhof, Stanford University	Neurexins and neuroligins: Synapses to autism.	Tony Zador
September		
Dr. Luis Parada, University of Texas Southwestern Medical Center	Modeling neural cancers in mice: Cell of origin and and other insights.	Scott Lowe
Dr. Peter Sarnow, Stanford University	Subversion of a liver-specific microRNA by hepatitis C virus.	Adrian Krainer
October		
Dr. Ihor Lemischka, Mount Sinai School of Medicine	Dissecting cell-fate regulation in stem cells.	Grigori Enikolopov
Dr. Mitchell Lazar, University of Pennsylvania School of Medicine	Nuclear receptors, epigenetics, and metabolism.	Terri Grodzicker
Dr. Carla Shatz, Stanford University	Moonlighting MHCI and brain circuit tuning.	Sydney Gary

	Title	Host
November		
Dr. James Lupski, Baylor College of Medicine	Genomic disorders: Mechanisms and assays for CNV associated with neuropsychiatric and other disease traits.	Dick McCombie
December		
Dr. John Diffley, Cancer Research, United Kingdom	Mechanism and regulation of DNA replication during the cell cycle and in response to DNA damage.	Leemor Joshua-Tor
Dr. Kim Orth, University of Texas, Southwestern Medical Center	Black death, black spot, black pearl: Tales of bacterial effectors.	Leemor Joshua-Tor
Dr. Bruce Spiegelman, Dana Farber Cancer Institute/Harvard Medical School	Transcriptional control of adipogenesis and energy homeostasis.	Terri Grodzicker

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	
Zhenyu Xuan (Zhang Lab)	Data mining through the Bioinformatics Shared Resource.
Dan Chitwood (Timmermans Lab)	Pattern formation via small RNA movement.
Jeffrey Rosenfeld (Zhang Lab)	The enigmatic nature of the "repressive" histone modification H3K9me3.
February	
Clint Whipple (Jackson Lab)	Evolution of convergent morphologies: Bract suppression in the grass family.
Prakash Kumar (Joshua-Tor Lab)	Turning ON transcription: A sugary tale revisited.
Johannes Zuber (Lowe Lab)	A path toward identifying the Achilles heel of chemoresistant leukemia.
Alexey Aravin (Hannon Lab)	Small RNA pathways in mouse germ line.
March	
Senthil Muthuswamy	Cell polarity: The nexus between morphogenesis and cancer.
Jason Bohland (Mitra Lab)	Neuroanatomy 2.0: Large-scale approaches to understanding brain organization.
Anirban Paul (Huang Lab)	When DNA is not enough: New targets for Rett syndrome protein.
April	
Santiago Jaramillo (Zador Lab)	What can auditory cortex tell about our expectations?
Agustin Chicas (Lowe Lab)	Dissecting the role of tumor suppressor pathways in cellular senescence.
Shilpi Paul (Mills Lab)	What makes CHD5 tick? Insights into its mechanism and partners.
October	
Alexei Aravin (Hannon Lab)	Arginine methylation of Piwi proteins and assembly of ribonucleoprotein granules in germ line.
Yimin Hua (Krainer Lab)	Antisense correction of SMN2 splicing in the CNS as a therapy for spinal muscular atrophy.
Eric Sawey (Powers Lab)	An oncogenomics-based in vivo cDNA screen pinpints novel therapeutic targets for liver cancer.
Nael Nadif Kasri (Van Aelst Lab)	Mind the gap: Rho GTPase signaling in X-linked mental retardation.

	Title
November	
Rafael Pagani (Zhong Lab)	Spacing effect in long-term memory induction.
Aftabul Haque (Tonks Lab)	Novel approaches to signal transduction-based therapy suggested by redox regulation of PTP1B.
Josh Dubnau	Memories of a fly: The rutabaga and the pack of dogs.
December	
Michael Feigin (Muthuswamy Lab)	Regulation of mammary gland morphogenesis and tumorigenesis by the polarity protein Scribble.
Alexei Koulakov	Olfactory space: Myth or reality?
Dick McCombie	Finding a needle in a haystack.

BANBURY CENTER

BANBURY CENTER
EXECUTIVE DIRECTOR'S REPORT

This was a busy year for the Banbury Center, with 20 science-related meetings and 555 participants. The proportion of U.S. participants was 79.7%, a number that has remained remarkably constant over the years. The U.S. participants came from 32 states, with the usual suspects leading the way (New York, Massachusetts, and Maryland). Foreign participants came from 22 countries, the majority from the United Kingdom. There tends to be a significant gender imbalance in Banbury Center meetings, but we did rather better in 2009 with more than 30% of participants being female.

Looking back at the decade just ended, there were 212 meetings at Banbury, with 6277 participants, an average of 31 participants per meeting. (These participants represent 4839 individuals; many came more than once in the period.) Seventy-seven percent of participants came from the U.S., and the percentage of female participants was 26%. The number of meetings each year ranged from a low of 17 in 2006 to a high of 26 in 2001.

To return to 2009, the year was characterized by a larger than usual number of meetings dealing with policy or the planning of research or promotion of a research area. Perhaps the most notable of these meetings was one held in December, on *Promoting Research on Severe Mental Illness.* The themes of the meeting were that finding the genes involved in severe mental disease is the most promising line of research and that the new generation of tools for large-scale genomics provides new opportunities for research on mental illness. Led by Jim Watson, Ed Scolnick, and Herb Pardes, participants reviewed the current state of genetic and genomics research and discussed how to advance this research. Participants prepared a "call to arms" that was published in the Policy Forum of *Science* (**327:** 1580–1581). Fittingly, support for the meeting was provided by foundations at the forefront of research on mental illnesses, including NARSAD–The Brain & Behavior Research Fund, World Heritage Foundation–Prechter Family Fund, and the Simons Foundation.

Robertson House

Aquatic Plants: Environment, Energy, and Evolution is another example of a meeting that combined research and policy. Duckweeds are familiar to us as the green "scum" floating on the surface of still bodies of water, such as ponds. They are tiny plants, without leaves or stems, and may or may not have rootlets. Duckweeds reproduce vegetatively as well as sexually and have the fastest known doubling time of flowering plants. Their advantages as experimental organisms are that they are easy to culture, have relatively small genomes, and can be transformed. Duckweeds are already used as food and in bioremediation, but participants in this meeting believe that the full potential of duckweed has yet to be realized. As a consequence, participants critically reviewed the biological and genetic properties of duckweed and examined the ways in which these properties could be exploited, and duckweed promoted, for biomass, biofuels, metabolic engineering, and bioremediation.

Continuing with this theme of meetings promoting policy, Banbury Center hosted a meeting of the International Steering Committee for Plant Genomics, an informal group comprised of funding organizations with a special interest in promoting genomic techniques for improving crops. The Committee, which includes scientists from Australia, Canada, France, Japan, Korea, the United Kingdom, and the United States, came to Banbury to develop a first draft of "a vision paper for the future of plant biology." Michael Gale, from the John Innes Institute in Norwich, England, was one of the participants, and we were saddened to learn of his death in July, 2009. Mike was one of the world's leading authorities on cereal genetics, and his research career was devoted to relieving world hunger through the improvement of cereal crops.

A notable scientific meeting was *Structural Variation in the Human Genome.* Structural changes in genomes have been known for many years. Studies of chromosomes revealed major rearrangements such as translocations, inversions, and insertions. The introduction of chromosome-banding stains in the 1960s revealed much smaller, intrachromosomal insertions and deletions. There was a further increase in resolution with the use of DNA probes and microarray techniques, and it became clear that changes could be as small as a few kilobases and were far more common in the human genome than had been thought. Indeed, copy-number variants (CNVs) are now recognized as one

Conference room

of the most common forms of genetic variation in human beings. CNVs have been associated with a range of human developmental disorders, including psychiatric disorders such as schizophrenia. Participants in this meeting discussed the mechanisms by which CNVs arise, their clinical consequences, and the development of diagnostics tests.

The Human Genome Project (HGP) was one of the great scientific enterprises of the 20th century, ranking with the Manhattan and Apollo Projects. The latter have been exhaustively documented, by the Department of Energy and by NASA, and we believe that the HGP warrants the same historical attention. We have initiated a program to document the history of the HGP and related sequencing projects. The first goal of this project is to create a comprehensive database of the locations, nature, and a description of materials relating to the HGP, whether held by scientists, academic institutions, foundations, or government departments. The discussion meeting *International Catalog for the History of the Human Genome Project* was held to examine how other organizations have assembled such databases, to review the proposed project, and to suggest amendments and modifications in the light of other experience. Participants included key players in the early development of the HGP, historians of science, and archivists working on similar projects. The discussions were most helpful for planning the next steps in our project.

The Boehringer Ingelheim Foundation promotes biomedical research by providing fellowships for graduate students performing research for a Ph.D. Each year, the Foundation holds a meeting in North America for their fellows, and 2009 marked the third occasion on which the Foundation has come to Banbury. It is always a pleasure to have scientists-in-training here, especially because the Foundation generously funds a special lecture, open to CSHL scientists. We were delighted to have Beth Shapiro (Pennsylvania State University) as our speaker. Beth had just been awarded a MacArthur Fellowship and, having heard her fascinating research, it was clear to see why she had been chosen.

Meetings participants and students taking 2009 summer courses have enjoyed the fruits of the refurbishment of Robertson House, which was carried out during the winter 2008–2009. The Robertson family has continued to help us in preserving Robertson House, and in particular, I would like to thank Victoria Linnartz, the granddaughter of Charles and Marie Robertson, for all of her hard work in enhancing the beauty of the house.

When I came to the Banbury Center, Bea Toliver was already here as assistant to the Banbury director and Katya Davey was hostess in Robertson House. It was immediately clear that we needed help and so, early in 1988, Ellie Sidorenko joined us in the Banbury office. Ellie was the first and only change in the Banbury Center staff for almost 20 years, and now, in the space of just a few years, Katya, Bea, and, in 2009, Ellie retired. Banbury has been very fortunate in recruiting three people who have immediately understood the Banbury style. Basia Polakowski took on the role of hostess in Robertson House in 2005, and, in 2009, Janice Tozzo became the Banbury Center assistant and was joined by Susanne Igneri later in the year.

As always, the operations of the Banbury Center depend on many people: Janice, Susanne, and Basia have already earned the thanks of meetings participants; Mike Peluso and the grounds crew continue to keep the Banbury grounds looking beautiful; Jon Parsons is indispensable in handling our AV needs; Connie Brukin takes interesting and artistic photographs of participants; and the staff of the Laboratory's Food Services and Housekeeping cope admirably with the very full schedule of Banbury meetings.

Jan Witkowski
Executive Director

BANBURY CENTER MEETINGS

NSF Workshop: A Vision for Plant Biology

March 1–4

FUNDED BY **Biotechnology and Biological Sciences Research Council (BBSRC), Deutsche Forschungsgemeinschaft (DFG), National Science Foundation, The Salk Institute for Biological Studies**

ARRANGED BY **J. Ecker,** The Salk Institute for Biological Studies
A. Millar, University of Edinburgh

The International Steering Committee on Plant Genomics is an informal group made up of funding organizations with a special interest in promoting genomic techniques in improving crops. Among other goals, the Committee is intended to facilitate communications between funding agencies in this area and promoting research collaborations. A small group of scientists drawn from participating countries (including Australia, Canada, France, Japan, Korea, the United Kingdom, and the United States) came to Banbury to discuss their perspectives of current and future challenges in the plant sciences and to develop an International Model for the Future of Plant Science. A draft report was prepared by the scientists at the conclusion of the meeting and presented at widely attended community meetings for comment during 2009. The final report will be published in 2010.

Orientation: **J. Ecker,** The Salk Institute for Biological Studies, San Diego, California
A. Millar, University of Edinburgh, Edinburgh, United Kingdom

SESSION 1

Scientists' presentations.

SESSION 2

Summarize speaker recommendations.
Draft outline.
Finalize writing assignments.

SESSION 3

Writing groups to draft sections.

SESSION 4

Writing groups to draft sections.
Progress review.

SESSION 5

Editing and compilation of draft document by cochairs.

SESSION 6

Wrap-up and development of powerpoint presentation for Cold Spring Harbor Laboratory meeting.

M. Bevan

S.-H. Lee, E. Dennis

J. Silverthorne, R. Bastow, K. Kistner, R. McKibbin

Neurobiology of Depression: From Molecules to Mood

March 22–25

FUNDED BY **AstraZeneca Pharmaceuticals, Hoffmann-LaRoche, Inc., Hope for Depression Research Foundation, Johnson & Johnson Pharmaceuticals Research, Lilly Research Laboratories, NIMH, Wyeth Pharmaceuticals**

ARRANGED BY **R. Hen,** Columbia University
B. McEwen, The Rockefeller University
R. Duman, Yale University
H. Manji, Johnson & Johnson Pharmaceutical Research

Depression is a devastating illness that affects 15%–20% of the population, resulting in enormous personal suffering and economic loss to society. Despite intensive research, the neurobiological mechanisms underlying the etiology and treatment of major depressive disorders have not been identified. The aim of this meeting was to bring together basic and clinical investigators to provide a comprehensive and integrated assessment of the current state of knowledge of depression research. Discussions covered the genetic, molecular, and cellular determinants of mood and depression in animal models and in humans, as well as the latest information from clinical studies on the circuitry, imaging and the treatment of mood disorders.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

Welcoming Remarks, Overview of the Agenda/ Schedule, and Objectives: **R. Hen,** Columbia University, College of Physicians & Surgeons, New York, New York

SESSION 1: Treatments and Models
Chairperson: R. Duman, Yale University School of Medicine, New Haven, Connecticut

H. Koenigsberg, Mount Sinai School of Medicine, New York: Does a psychosocial perspective have something to offer neurobiological research in depression and vice versa?

D. Charney, Mount Sinai School of Medicine, New York: Therapeutic effects of ketamine in treatment-resistant depression.

H. Manji, Johnson & Johnson Pharmaceutical Research & Development, Titusville, New Jersey: Targeting synaptic and cellular plasticity cascades to develop improved therapeutics.
C. Nemeroff, Emory University School of Medicine, Atlanta, Georgia: Early life trauma in major depression: A distinct endophenotype.
B. McEwen, The Rockefeller University, New York: Depression, adaptive plasticity, and resilience: Insights from animal models.

SESSION 2: Imaging/Circuitry
Chairperson: H. Manji, Johnson & Johnson Pharmaceutical Research & Development, Titusville, New Jersey

Y. Sheline, Washington University School of Medicine, St. Louis, Missouri: Resting state connectivity studies in major depression.
S. Maier, University of Colorado, Boulder, Colorado: Role of the medial prefrontal cortex in resilience and vulnerability.
M. George, Medical University of South Carolina, Charleston: Noninvasively modulating prefrontal cortical–subcortical networks to treat depression: The current evidence for TMS and future directions.
H. Mayberg, Emory University School of Medicine, Atlanta, Georgia: Deep brain stimulation for treatment-resistant depression.
K. Disseroth, Stanford University, Stanford, California: Development and application of optical technologies for probing depression.

SESSION 3: Genes and Development
Chairperson: R. Hen, Columbia University, College of Physicians & Surgeons, New York

K. Kendler, Virginia Commonwealth University, Richmond: The genetic epidemiology of major depression.
J. Gingrich, Columbia University, New York: Role of developmental serotonin signaling in adult affective function.
K. Ressler, Emory University, Atlanta, Georgia: BDNF in prefrontal cortex, amygdala, and hippocampus required for extinction of aversive memories.
M. Meaney, McGill University, Montreal, Canada: Epigenetic mechanisms for the environmental programming of gene expression.
E. Nestler, Mount Sinai School of Medicine, New York: Epigenetic mechanisms of depression.

B. McEwen, R. Hen

SESSION 4: Stress and Sex
Chairperson: H. Akil, University of Michigan, Ann Arbor

R. Duman, Yale University School of Medicine, New Haven, Connecticut: Neurotrophic factors in the pathophysiology and treatment of depression.
N. Sousa, University of Minho, Braga, Portugal: Role of neurogenesis in depression.
W. Carlezon, Harvard Medical School–McLean Hospital, Belmont, Massachusetts: Stress, behavior, and hippocampal neurogenesis.
A. Miller, Emory University School of Medicine, Atlanta, Georgia: Inflammation and its discontents: Role of cytokines in the pathophysiology of depression.
P. Schmidt, National Institutes of Health/NIMH, Bethesda, Maryland: Reproductive endocrinology and mood disorders in women.
T. Schors, Rutgers University, Piscataway, New Jersey: Why are females so smart and yet so susceptible to stress?

SESSION 5: New Directions
Chairperson: B. McEwen, The Rockefeller University, New York

A. Lewy, Oregon Health Sciences University, Portland: Circadian misalignment component of sleep and mood disorders.
R. Hen, Columbia University, College of Physicians & Surgeons, New York: Mechanisms underlying the response to antidepressants: Responders versus nonresponders.
H. Akil, University of Michigan, Ann Arbor: Novel molecular targets in mood disorders.
Z. Nahas, Medical University of South Carolina, Charleston: Oxytocin for depression.
M. Solms, University of Cape Town, Roundebosch, South Africa: Depression and separation distress.
L. Brady and R. Nakamura, National Institutes of Health/NIMH, Bethesda, Maryland: NIH initiatives.

New Developments in Fragile X Syndrome: From Basic Mechanisms to Therapeutics

April 5–8

FUNDED BY **NIHM grant to the University of Illinois**

ARRANGED BY **K. Huber,** University of Texas Southwestern Medical Center
F. Gasparini, Novartis Pharma AG
W.T. Greenough, University of Illinois
E. Berry-Kravis, Rush University Medical Center
K. Clapp, FRAXA Research Foundation

There has been much progress in the past years in several areas of research on Fragile X, but there is still much to be learned. This meeting focused on the basic mechanisms of FMRP function, the consequences of its loss on synaptic and circuit function, and therapies for treatment. Some questions that were examined included: How does FMRP regulate translation and processing of its associated mRNAs? How is neuronal circuit function altered in Fragile X Syndrome and can this explain the behavioral symptoms of the disease?

Introductory Remarks: **J.A. Witkowski,** Banbury Center
Opening Comments: **K. Clapp,** FRAXA Research Foundation, Newburyport, Massachusetts

SESSION 1: Molecular Functions of FMRP and Related Proteins
Chairperson: D. Nelson, Baylor College of Medicine, Houston, Texas

H. Moine, Institute de Genetique, Illkirch, France: FMRP in mRNA metabolism: From RISC to dendritic mRNA control.

C. Bagni, K.U. Leuven University, Belgium: Developmental changes of the FMRP-BC1 interaction alter the regulation of synaptic translation.

S. Vasudevan, Yale University, New Haven, Connecticut: Translational regulation by microRNPs.
J. Darnell, The Rockefeller University, New York: Identification of neuronal RNA ligands of FMRP by cross-linking–IP analysis.
J. Richter, University of Massachusetts Medical School, Worcester: RNA processing and synaptic plasticity.
P. Lombroso, Yale University School of Medicine, New Haven, Connecticut: Taking STEPs to improve cognition: Role of STEP in Fragile X syndrome.

SESSION 2: Synaptic Mechanisms of FMRP Function and Alterations in Fragile X Syndrome
Chairperson: W.T. Greenough, University of Illinois, Urbana

G. Bassell, Emory University, Atlanta, Georgia: FMRP and miRNA-mediated translation regulation at synapses.
P. Vanderklish, Scripps Research Institute, La Jolla, California: Kinase and phosphatase regulatory protein abnormalities in the Fmr1 KO.
K. Huber, University of Texas Southwestern Medical Center, Dallas: FMRP regulation of mGluR-dependent long-term depression.
S. Zukin, Albert Einstein College of Medicine, Bronx, New York: Dysregulation of mTOR signaling in a mouse model of Fragile X.
I. Weiler, University of Illinois, Urbana Champaign: A novel presynaptic defect in FXS.
T. Price, University of Arizona, Tucson: Axonal plasticity, FMRP, and pain.
M. Zhuo, University of Toronto, Ontario, Canada: Regulation of FMRP in prefrontal cortex.
M. Bear, Massachusetts Institute of Technology, Cambridge: Hypersensitivity to, not hyperactivity of, mGluR5 in Fragile X.
S. Chattarji, Tata Institute of Fundamental Research, Bangalore, India: Characterization and reversal of synaptic defects in the amygdala in a mouse model of Fragile X syndrome.
B. Oostra, Erasmus University, Rotterdam, The Netherlands: Effects of mGluR antagonists on neurons.

SESSION 3: Circuit Function and Development in Fragile X Syndrome
Chairperson: K. Huber, University of Texas Southwestern Medical Center, Dallas

K. Broadie, Vanderbilt University & Medical School, Nashville, Tennessee: *Drosophila* model of Fragile X: Brain circuit function.
D. Nelson, Baylor College of Medicine, Houston, Texas: Developmental requirements of FMRP.
P. Kind, University of Edinburgh, United Kingdom: Role of FMRP in early postnatal development of the cerebral cortex.
J. Gibson, University of Texas Southwestern Medical Center, Dallas: Alterations in neocortical connectivity and circuit function in the Fmr1 KO mouse.
M. Avoli, McGill University, Montreal, Canada: Tonic inhibition and Fragile X syndrome.
R. Wong, SUNY–Downstate Health Science Center, Brooklyn, New York: Plasticity mechanisms for mGluR-mediated epileptiform activity in Fragile X mice.
L. Kaczmarek, Yale University School of Medicine, New Haven, Connecticut: FMRP and sodium-activated potassium channels.

SESSION 4: Therapeutics in Animal Models of Fragile X Syndrome and Patients
Chairperson: M. Tranfaglia, FRAXA Research Institute, Newburyport, Massachusetts

F. Bolduc, University of Alberta, Edmonton, Canada: Fragile X flies and memory: Therapeutic approaches.
W. Spooren, F. Hoffmann-LaRoche Ltd., Basel, Switzerland: Translational aspects of Fragile X.
F. Gasparini, Novartis Pharma AG, Basel, Switzerland: MGluR5 antagonists as antihyperalgesic in models for inflammatory pain.
R. Hagerman, University of California Davis Health System, Sacramento: Minocycline treatment in Fragile X syndrome.
E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois: Pilot measures of inhibition and executive function for clinical trials in FXS.
S. Jacquemont, University Hospital, Lausanne, Switzerland: Phase-2 clinical trials of a specific mGluR5 antagonist in Fragile X syndrome.
C.B. Smith, National Institutes of Health/NIMH, Bethesda, Maryland: Fragile X permutation: Morphological, behavioral, and neurochemical effects in a mouse model.

W. Spooren

Searching for Principles Underlying Memory in Biological Systems

April 12–15

FUNDED BY **The Swartz Foundation**

ARRANGED BY **W.A. Suzuki**, New York University
S. Fusi, Columbia University

This workshop brought together experimentalists and theoreticians studying both medial temporal lobe functions and prefrontal functions, as well as their interactions. Participants included experimentalists working on questions of synaptic plasticity and systems-level behavioral neurophysiology, as well as human neuropsychologists. The goal was to encourage interaction and discussion between theoreticians and experimentalists working at all of these different levels of analysis.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introduction: Why this meeting? **W.A. Suzuki,** New York University, New York
S. Fusi, Columbia University, New York

SESSION 1: Functional Organization of the Medial Temporal Lobe: Views From Across Different Experimental Systems
Chairperson: L. Davachi, New York University, New York

H. Eichenbaum, Boston University, Boston, Massachusetts: How does the hippocampus integrate "what" and "where" information?
J.T. Wixted, University of California, San Diego, La Jolla: Interpreting memory-related activity in the medical temporal lobe.
Y. Dudai, Weizmann Institute of Science, Rehovot, Israel: Predicting not to predict too much: How the cellular machinery of memory anticipates the uncertain future.
W.A. Suzuki, New York University, New York: Task-dependent patterns of medial temporal lobe activity.
P. Dayan, University College London, United Kingdom: Episodic control: Singular recall and optimal actions.

SESSION 2: Functional Organization of the Medial Temporal Lobe: Views from Across Different Experimental Systems
Chairperson: H. Eichenbaum, Boston University, Boston, Massachusetts

D.L. Shachter, Harvard University, Cambridge, Massachusetts: Episodic simulation of future events and the medial temporal lobe.
D. Shohamy, Columbia University, New York: Striatal and hippocampal contributions to different forms of learning.
L. Davachi, New York University, New York: Memory signals in the human medial temporal lobe.
G. Buzsaki, Rutgers, The State University of New Jersey, Newark: Cell assembly sequences in the service of memory.
C. Stark, University of California, Irvine: Pattern separation in the human hippocampus.

SESSION 3: Hippocampal Functions: Theoretical and Experimental Views
Chairperson: G. Buzsaki, Rutgers, The State University of New Jersey, Newark

N. Burgess, University College London, United Kingdom: Neural mechanisms of spatial memory.
L. Colgin, Kavli Institute for Systems Neuroscience, Trondheim, Norway: High and low frequencies of γ oscillations serve as discrete communication channels in the hippocampus.
J.J. Knierim, Johns Hopkins University, Baltimore, Maryland: Medial and lateral entorhinal inputs to the hippocampus.
M.A. Wilson, Massachusetts Institute of Technology, Cambridge: Memory reactivation in the hippocampus.
S. Fusi, Columbia University, New York: Memories on multiple timescales: The importance of heterogeneity and memory transfer.

G. Buzsaki

SESSION 4: Working Memory
Chairperson: S. Grant, Wellcome Trust Sanger Institute, Hinxton, United Kingdom

E.K. Miller, Massachusetts Institute of Technology, Cambridge: Brain rhythms and short-term memory.
X.J. Wang, Yale University School of Medicine, New Haven, Connecticut: Slow reverberation mechanism of working memory.
S. Ganguli, University of California, San Franciso: Short-term sequence memory in neuronal networks.
A. Treves, SISSA–Cognitive Neuroscience, Trieste, Italy: Creative latching dynamics in simplified cortical networks.
M.V. Tosodyks, Weizmann Institute of Science, Rehovot, Israel: Synaptic theory of working memory.

N. Burgess, C. Stark

SESSION 5: Synaptic Plasticity: Theory and Experiments
Chairperson: W.A. Suzuki, New York University, New York

L.F. Abbott, Columbia University, New York: Chemical kinetics and memory.
S. Grant, Wellcome Trust Sanger Institute, Hinxton, United Kingdom: Synapse complexity.
A. Maffei, SUNY, Stony Brook, New York: Plasticity of inhibition in visual cortex.
S. Wang, Princeton University, Princeton, New Jersey: Elements of synaptic learning rules.
M.A. Hauser, University College London, United Kingdom: How do the properties of dendrites influence synaptic plasticity and memory storage?

Y. Dudai

Molecular Biology of Sirtuins

April 26–29

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **L.P. Guarente,** Massachusetts Institute of Technology
D.A. Sinclair, Harvard Medical School

In the 1990s, the Sir2 protein was identified as a key regulator of lifespan in budding yeast. Since then, it has become increasingly clear that the Sir2 family proteins are highly conserved enzymes that mediate many of the health benefits of calorie restriction. Sirtuins are becoming increasingly appreciated for their potential in treating diverse diseases, from neurodegeneration to Type II diabetes. The modulation of sirtuins by metabolites such as NAD^+ and nicotinamide, by environmental changes such as DNA damage, diet and exercise, and most recently by small drug-like molecules, has further increased interest in sirtuins. Participants in this meeting discussed new directions for research on sirtuins and identified how sirtuins can be used for improving human health.

Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Metabolism 1
Chairperson: D.A. Sinclair, Harvard Medical School, Boston, Massachusetts

D. Accili, Columbia University, New York: Metabolic actions of Sirt1.
J. Auwerx, Ecole Polytechnique Federal de Lausanne, Switzerland: A cofactor network to control metabolism.
N. Barzilai, Albert Einstein College of Medicine, Bronx, New York: Central effects of Sirt1 on peripheral glucose homeostasis.
R. de Cabo, National Institutes of Health/NIA, Baltimore, Maryland: Health and longevity consequences of activating SIRT1.

M.Y. Donath, University Hospital Zurich, Switzerland: SIRT1 in type-1 diabetes and autoimmune/autoinflam-matory diseases.
S.-I. Imai, Washington University School of Medicine, St. Louis, Missouri: Systemic regulation of metabolism and aging by SIRT1 and Nampt-mediated NAD biosynthesis.
C. Deng, National Institutes of Health/NIDD, Bethesda, Maryland: Sirt6 in glucose and fat metabolism.

SESSION 2: Neuro-Cancer
Chairperson: D. Accili, Columbia University, New York

A. Brunet, Stanford University, Stanford, California: Sirt1 and Fox03 in adult neural stem cells.
R. Coppari, University of Texas Southwestern Medical Center, Dallas: Brain SIRT1: A novel target to treat diet-induced metabolic dysfunctions?
L.P. Guarante, Massachusetts Institute of Technology, Cambridge: Sirtuins and disease.
L.-H. Tsai, Massachusetts Institute of Technology, Cambridge: Role of SIRT1 in neuroprotections and cognition.
E.N. Chini, Mayo Clinic, Rochester, Minnesota: Role of the NADase CD38 and the nuclear protein DBC1 as regulators of SIRT1 and metabolism.
Z. Lou, Mayo Clinic, Rochester, Minnesota: A c-Myc/SIRT 1 feedback loop regulates cell growth and transformation.

SESSION 3: Metabolism 2
Chairperson: A. Brunet, Stanford University, Stanford, California

S.-J. Lin, University of California, Davis: Sirtuins, NAD metabolism, and calorie restriction: Insight from *S. cerevisiae.*
M.W. McBurney, Ottawa Health Research Institute, Ontario, Canada: Roles of FMRP in neuronal architecture development and synaptogenesis.
T. Nystrom, CMB Gothenborg University, Gothenborg, Sweden: Global genetic interaction network of Sir2 and damage segregation.
V. Sartorelli, National Institutes of Health/NIAMS, IRP, Bethesda, Maryland: Roles of SIRT1 in skeletal muscle.
K. Irani, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania: Nitroso-redox regulation of SIRT1.
P. Sassone-Corsi, University of California, Irvine: SIRT and circadian clock.

SESSION 4: Metabolism 3 and Drugs
Chairperson: S.-J. Lin, University of California, Davis

D.A. Sinclair, Harvard Medical School, Boston, Massachusetts: Genes and small molecules that modulate sirtuins.
Y. Suh, Albert Einstein College of Medicine, Bronx, New York: Genetic variation in sirtuins and disease.
L. Bordone, Novartis Institute for Biomedical Research, Cambridge, Massachusetts: Therapeutic opportunities for sirtuins.
G.P. Vlasuk and C. Westphal, Sirtris, Cambridge, Massachusetts: New therapeutic opportunities for pharmacological modulators of sirtuin activity.

SESSION 5: SIRT2–7
Chairperson: L.P. Guarante, Massachusetts Institute of Technology, Cambridge

M. Haigis, Harvard Medical School, Boston, Massachusetts: Understanding the impact of sirtuins on mitochondrial metabolism.
A.A. Sauve, Weill Cornell Medical College, New York: Enzymology and chemical reactivity of SIRT5 and SIRT6.
Q. Tong, Baylor College of Medicine, Houston, Texas: Functions of SIRT2 and SIRT3.

A. Brunet, S.-I. Imai, P. Sassone-Corsi

The First NIMH-sponsored Brain Camp

April 29–May 2

FUNDED BY **National Institute of Mental Health**

ARRANGED BY **M. Akil,** National Institute of Mental Health
T.R. Insel, National Institute of Mental Health

There is a need to link psychiatry training to neuroscience. Although psychiatric disorders such as schizophrenia and depression are regarded as brain disorders, current training programs in psychiatry include very little of the findings of modern neuroscience. This NIMH-sponsored "brain camp" was a first attempt to see whether this situation can be rectified by providing psychiatry residents with an intense exposure to the world of cognitive neuroscience. To this end, more than 20 psychiatry residents and 17 speakers participated in in-depth discussions of a variety of topics.

Introduction and Charge: **T.R. Insel,** National Institute of Mental Health, Bethesda, Maryland

SESSION 1: Genetics

F. McMahon, National Institute of Mental Health, Bethesda, Maryland

M.W. State, Yale University School of Medicine, New Haven, Connecticut

SESSION 2: Social Neuroscience: The social brain

R. Adolphs, California Institute of Technology, Pasadena
T.R. Insel, National Institute of Mental Health, Bethesda, Maryland

S. Wang

SESSION 3: Developmental Neurobiology/Schizophrenia

S.A. Anderson, Weill Medical College of Cornell University, New York
D.A. Lewis, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

Discussion with the organizers: Teaching neuroscience in medical school and during psychiatry training; what's missing?

R. Caceda

D. Ross

SESSION 4: The Neurobiology of Affect, Emotion, and Affective Disorders

M. Davis, Emory University, Atlanta, Georgia
H. Akil, University of Michigan, Ann Arbor
K. J. Ressler, Emory University, Atlanta, Georgia

SESSION 5: Cognitive Neuroscience

C. Carter, University of California Davis, Sacramento
J.D. Cohen, Princeton University, Princeton, New Jersey
D. Barch, Washington University, St. Louis, Missouri

T. Insel, A. Yusim

Special Presentation: Molecular and Cellular Tools for Studying and Perturbing Brain Circuits
K. Deisseroth, Stanford University, Stanford

SESSION 6: Cognitive Neuroscience II

J. Wallis, University of California, Berkeley
M. Frank, Brown University, Providence, Rhode Island

SESSION 7: Effects of Pre- and Postnatal Experiences on Adult Stress Response and Disease Vulnerability

E.J. Nestler, Mount Sinai School of Medicine, New York
F. Champagne, Columbia University, New York

J. Coughlin, M. Nakic

International Catalog for the History of the Human Genome Project

June 16–17

FUNDED BY **The Wellcome Trust**

ARRANGED BY **L. Pollock,** Library and Archives, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

The Human Genome Project (HGP) was one of the great scientific enterprises of the 20th century, and we have initiated a program to document the history of the HGP and related sequencing projects. The first goal of this project is to create a comprehensive database of the locations, nature, and description of materials relating to the HGP, whether held by scientists, academic institutions, foundations, or government departments. This discussion meeting was held to examine how other organizations have assembled such databases, to review the proposed project, and to suggest amendments and modifications in the light of other experience.

Welcome: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introductory Remarks: **L. Pollock,** Library and Archives, Cold Spring Harbor Laboratory

SESSION 1: Case Studies

J. Anderson, Niels Bohr Library and Archives, American Institute of Physics, College Park, Maryland: Center for History of Physics at the American Institute of Physics.
P. Theerman, NLM History of Medicine, Bethesda, Maryland: National Library of Medicine's Profiles in Science.
J. Van Oudenaren, World Digital Library, Library of Congress, Washington, D.C.: World Digital Library.
R. Moore, Data Intensive Cyber Environments, Renaissance Computing Institute, Chapel Hill, North Carolina: Managing and integrating diverse data sets.

M. Olson, J.D. Watson

SESSION 2: About the Project

M. Pollock, Library and Archives, Cold Spring Harbor Laboratory: Immediate and future goals.

SESSION 3: Planning the Next Steps: What Should We Consider?

Types and Sources of Materials
What period should be covered?
What should be collected?
Where is it?

How Much is There?
Organization—How to do it?
Project plan
Phases
Collaborations

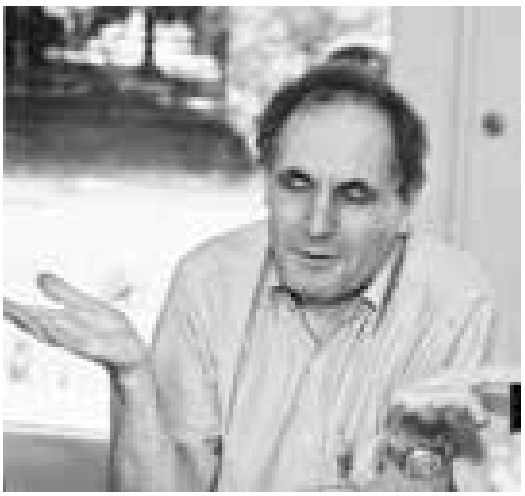
Funding: How Can We Fund the Project?
Where from?
What about long-term support?
What working groups would be useful?
Group I: Technical issues relating to managing and integrating data.
Group II: Identifying collections and owners of relevant materials.
Group III: Finance.

SESSION 4: Summary and Conclusions

Personal Remarks: J.D. Watson, Cold Spring Harbor Laboratory

E. Carlson

A. Patrinos, C. DeLisi



J. Weissenbach

Arbuscular Mycorrhizal Symbioses and Their Impact on Plant Nutrition

September 8–11

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M. Harrison,** Cornell University
U. Paszkowski, Université de Lausanne

The arbuscular mycorrhizal (AM) symbiosis is a widespread plant–fungal interaction that occurs between roots of terrestrial plants and Glomeromycotan fungi. This association has received considerable scientific attention because of (1) the nutritional benefit it confers on the plant by improving access to otherwise limiting sources of nutrients, (2) its widespread occurrence among extant plants and significance in terrestrial ecosystems, and (3) its ancestral role in the evolution of symbiosis signaling pathways. This discussion meeting focused on AM symbioses and their impact on plant nutrition and brought together key researchers from the AM symbiosis, plant mineral nutrition, and root architecture fields.

Introductory and Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
M. Harrison, Boyce Thompson Institute, Cornell University, Ithaca, New York
U. Paszkowski, Université de Lausanne, Lausanne, Switzerland

SESSION 1: Signaling and Development of Arbuscular Mycorrhizal Symbiosis
Chairperson: M. Parniske, University of Munich, Munich, Germany

P. Young, University of York, United Kingdom: The glomus genome: What we have learnt so far?
G. Becard, University of Toulouse, France: Early signaling in the AM symbiosis from the lab to the field.
M. Parniske, University of Munich, Germany: Signal transduction in symbiosis.
G. Oldroyd, John Innes Center, BBSRC, United Kingdom: Early signaling events during the establishment of the mycorrhizal association.

SESSION 2: Phosphorus and Nitrogen Sensing and Signaling
Chairperson: S. Abel, University of California, Davis

M. Udvardi, Samuel Roberts Nobleel Foundation, Ardmore, Oklahoma: HERMES: A Medicago gene required for both AM and nitrogen-fixing/rhizobial symbioses.
S. Abel, University of California, Davis: Phosphate sensing in root development.
J. Paz-Ares, Centro National de Biotechnologia, Madrid, Spain: Phosphate starvation signaling in *Arabidopsis.*
B. Forde, Lancaster University, United Kingdom: Nitrogen signaling and the modulation of root architecture.

SESSION 3: Root Architecture and Mineral Nutrient Acquisition
Chairperson: D. Schachtman, Donald Danforth Plant Science Center, St. Louis, Missouri

E. Nielsen, University of Michigan, Ann Arbor: Polarized membrane trafficking during root hair growth.
C. Hardtke, Université de Lausanne, Lausanne, Switzerland: Natural genetic variation.
J. Lynch, Pennsylvania State University, University Park: Root architecture and AMs.

SESSION 4: Mineral Nutrient Acquisition in AM Symbiosis
Chairperson: M. Harrison, Boyce Thompson Institute, Cornell University, Ithaca, New York

I. Jakobsen, Technical University of Denmark, Roskilde, Denmark: Functional analysis of Pi uptake pathways by means of VIGS.
D. Schachtman, Donald Danforth Plant Science Center, St. Louis, Missouri: Response of mycorrhizal and nonmycorrhizal tomato roots to nutrient enriched soil patches.
E. Neumann, Institute of Vegetable and Ornamental Crops, Grossbeeren, Germany: Exploitation of different soil nitrogen resources by the extraradial mycelium of arbuscular mycorrhizal fungi.
Y. Shachar-Hill, Michigan State University, East Lansing: Movement and metabolism of carbon, nitrogen, and sulfur in the AM symbiosis.
D. Reinhardt, University of Fribourg, Fribourg, Switzerland: Regulation of AM symbiosis by nutrients.
M. Harrison, Boyce Thompson Institute, Cornell University, Ithaca, New York: Phosphate transport in AM symbiosis.

SESSION 5: Functional Diversity in AM Symbiosis
Chairperson: U. Paszkowski, Université de Lausanne, Lausanne, Switzerland

J. Bever, Indiana University, Bloomington: Preferential allocation of plant photosynthate and the maintenance of the AM mutualism.
D. Janos, University of Miami, Coral Gables, Florida: Evolution of dependence upon, responsiveness to, and effectiveness of AM.
S. Kaeppler, University of Wisconsin, Madison: Variation among maize lines for mycorrhizal responsiveness under P stress.
U. Paszkowski, Université de Lausanne, Lausanne, Switzerland: Molecular genetics of the AM symbiosis in cereals.

U. Paszkowski

From Infection to Neurometabolism: A Nexus for CFS

September 13–16

FUNDED BY **National Institutes of Health and the CFIDS Association of America**

ARRANGED BY **S. Vernon,** CFIDS Association of America
E. Hanna, NIH, Office of Research on Women's Health

Chronic fatigue and widespread pain are common physical symptoms and unfortunately are the most likely to remain unexplained. Chief among illnesses characterized by medically unexplained chronic fatigue and pain is chronic fatigue syndrome (CFS), afflicting at least 4 million American adults. Despite more than 20 years of CFS research and more than 5000 peer-reviewed biomedical publications detailing infection, genetic polymorphisms, and brain metabolism in CFS, there is still no evidence-based diagnosis and treatment. The objective of this workshop was to lay the foundation for an expanded CFS research network that will work toward evidence-based objective diagnosis and treatment of CFS.

Introductory and Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Gathering and Integration of Information to Identify Biomarkers for CFS Diagnosis and Treatment
Chairpersons: S. Vernon, CFIDS Association of America, Charlotte, North Carolina, and
E. Hanna, NIH, Office of Research on Women's Health, Bethesda, Maryland

K. McCleary, CFIDS Association of America, Charlotte, North Carolina: Supporting CFS research through advocacy.

E. Hanna, NIH, Office of Research on Women's Health, Bethesda, Maryland: Establishing and supporting a CFS research network.

L. Bateman, The Fatigue Consultation Clinic, Salt Lake City, Utah: The minimal clinical information required in a research network.

L. Jason, DePaul University, Chicago, Illinois: Importance of defining CFS for research studies.

S. Vernon, CFIDS Association of America, Charlotte, North Carolina: The minimal laboratory information required in a research network.

B. Mishra, New York University, New York: Computational possibilities of a research network.

S. Srivastava, National Cancer Institute, Bethesda, Maryland:

Establishing an evidence-based biomarker research network.
K. Helmer, Massachusetts General Hospital, Charleston: Creating a research network using BIRN resources.

SESSION 2: Infectious and Immunologic Biomarkers of CFS
Chairpersons: S. Vernon, CFIDS Association of America, Charlotte, North Carolina, and
E. Hanna, NIH, Office of Research on Women's Health, Bethesda, Maryland

M. Hornig, Columbia University, New York: Chronic viruses as biomarkers.
B. Katz, Children's Memorial Hospital, Chicago, Illinois: EBV: Cause, trigger, or sustain CFS.
B. Huber, Tufts University School of Medicine, Boston, Massachusetts: Endogenous retroviruses: Cause, trigger, or sustain CFS.
R. Engler, Walter Reed Army Medical Center, Washington, D.C.: Vaccines and CFS.
T. Theoharides, Tufts University School of Medicine, Boston, Massachusetts: Mast cells and CFS.
K. Light, University of Utah Health Sciences Center, Salt Lake City: Peripheral blood biomarkers in CFS.
M. Fletcher, University of Miami School of Medicine, Miami, Florida: Neuroimmune biomarkers in CFS.

Evidence evaluation and summary sessions of infection and immunity presentations
S. Raj, A. Vincent, N. Klimas, T. Komaroff, L. Royster, C. Bausch, S. Vernon, E. Hanna, K. McCleary, S. Yale, E. Aslakson, B. Evengard

SESSION 3: ANS and CNS Biomarkers of CFS
Chairpersons: S. Vernon, CFIDS Association of America, Charlotte, North Carolina, and
E. Hanna, NIH, Office of Research on Women's Health, Bethesda, Maryland

P. Rowe, Johns Hopkins University, Baltimore, Maryland: Orthostatic stability and instability.
I. Biaggioni, Vanderbilt University, Nashville, Tennessee: Biomarkers of blood pressure regulation.
M. Medow, New York Medical College, Valhalla: Cerebral blood flow and autoregulation.
J. Stewart, New York Medical College, Hawthorne: Blood flow biomarkers in CFS.
D. Shungu, Weill Medical College of Cornell University, New York: Brain metabolic correlates of CFS.
D. Cook, University of Wisconsin, Madison: Brain function as indicated by fMRI in CFS.
B. Natelson, Beth Israel Medical Center, New York: Neurologic biomarkers in CFS.

SESSION 4: Future Directions
Chairpersons: K. McCleary, CFIDS Association of America, Charlotte, North Carolina, and
L. Royster, DePaul University, Chicago, Illinois

A. Kogelnik, Open Medicine Institute, Mount View, California: Electronic medical record and genetics as biomarkers of CFS.
J. Baraniuk, Georgetown University, Washington, D.C.: Proteomic biomarkers of CFS.
G. Broderick, University of Alberta, Edmonton, Canada: Genomic identification of dysfunctional cell subsets as biomarkers.
S. Shukla, Marshfield Clinic Research Foundation, Marshfield, Wisconsin: Metagenomic approach to investigate microbiological markers.

Workgroup meets to evaluate evidence and summarize sessions of ANS, CNS, and omic presentations
S. Raj, A. Vincent, N. Klimas, T. Komaroff, L. Royster, C. Bausch, S. Vernon, E. Hanna, K. McCleary, S. Yale, E. Aslakson, B. Evengard

SESSION 5: Evaluation and Summary Reports
Chairpersons: S. Vernon, CFIDS Association of America, Charlotte, North Carolina, and
E. Hanna, NIH, Office of Research on Women's Health, Bethesda, Maryland

Y. Setty, Microsoft Research Cambridge, Cambridge, United Kingdom: Can a research network help realistic modeling of CFS?
A. Komaroff, Harvard Medical School, Boston, Massachusetts: Infectious agents as CFS biomarkers.
N. Klimas, University of Miami, Miami, Florida: Immunologic and omic biomarkers of CFS.
S. Raj, Vanderbilt University, Nashville, Tennessee: ANS and CNS biomarkers of CFS.

Research Network Next Steps:
E. Hanna, NIH, Office of Research on Women's Health, Bethesda, Maryland
S. Vernon, CFIDS Association of America, Charlotte, North Carolina

Epigenetic Inheritance, Gene Regulation, and Plant Development

September 20–23

FUNDED BY **The Cold Spring Harbor–Pioneer Collaborative Research Program**

ARRANGED BY **R. Martienssen,** Cold Spring Harbor Laboratory
S. Tingey, DuPont Experimental Station

This meeting fulfilled two functions. The first was that it provided an opportunity for participants in the Cold Spring Harbor Laboratory–DuPont-Pioneer collaboration to meet one another and to exchange data, information, and ideas, and to review progress. Second, epigenetics is of special interest in plant science, and one day of the meeting was devoted to a minisymposium on the topic with invited speakers drawn from outside the collaboration.

Introductory and Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Epigenetics and Functional Genomics
Chairperson: J.A. Rafalski, DuPont Experimental Station, Delaware

R. Martienssen (R. Schwab), Cold Spring Harbor Laboratory: Efficiency of artificial miRNA.
M. Aukerman, DuPont Experimental Station, Wilmington, Delaware: miRNAs for translational silencing.
B. Meeley, Pioneer Hi-Bred International, Johnston, Iowa: Activation tagging in maize and other updates.
M. Tanurdzic, Cold Spring Harbor Laboratory: Epigenetic variation.
W. McCombie, Cold Spring Harbor Laboratory: Next-generation sequencing.
J. Hicks and J. Kendall, Cold Spring Harbor Laboratory: Hybrid capture bisulfite sequencing.
M. Regulski, Cold Spring Harbor Laboratory: Hybrid capture of maize DNA/discussion.

SESSION 2: Gene Expression and Development
Chairperson: D. Jackson, Cold Spring Harbor Laboratory

J. Tisdall, DuPont Experimental Station, Wilmington, Delaware: Whole transcriptome work.
A. Eveland, Cold Spring Harbor Laboratory: Analysis of solexa transcriptome data.
V. Llaca, Pioneer Hi-Bred International, Johnston, Iowa and S. DesChamps, Dupont Experimental Station, Wilmington, Delaware: SBS technology.
P. Bommert, Cold Spring Harbor Laboratory: Positional cloning of fasciated ear genes.
M. Guo, Pioneer Hi-Bred International, Johnston, Iowa: Regulation of organ size in maize.
D. Jackson, Cold Spring Harbor Laboratory: Fluorescent reporter lines in maize.
M. Dotto, Cold Spring Harbor Laboratory: Leaf patterning by laxmidrib and tasiRNA pathways.

SESSION 3: Genomes and Epigenomes in Plant Development
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

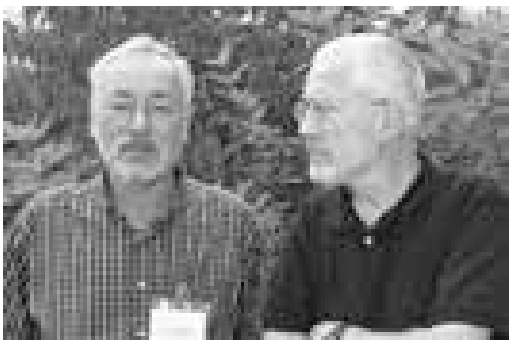
D. Ware, Cold Spring Harbor Laboratory: Sequencing the maize genome.
A. Rafalski, DuPont Experimental Station, Wilmington, Delaware: Genome technologies.
N. Springer, University of Minnesota, St. Paul: Links between genomic structure variation and epigenetic variation in maize.
M. Timmermans, Cold Spring Harbor Laboratory: Mutations in the maize tasiRNA pathway affect multiple developmental processes.
Z. Lippman, Cold Spring Harbor Laboratory: Inflorescence architecture, flowering, and heterosis.
V. Chandler, Gordon and Betty Moore Foundation, Palo Alto, California: Paramutation.

SESSION 4: Epigenetic Inheritance
Chairperson: M. Auckerman, DuPont Experimental Station, Delaware

V. Colot, CNRS–École Normale Supérieure, Paris, France: The other side of genetics: Epigenetics across generations.
J. Paszkowski, Unversity of Geneva, Geneva, Switzerland: Role of methylation in transgenerational inheritance.
E. Richards, Cornell University, Ithaca, New York: Epigenetic variation or resistance gene variation/instability.
R. Martienssen, Cold Spring Harbor Laboratory: Reprogramming of transposons in pollen.
J.P. Vielle-Calzada, National Laboratory of Genomics for Biodiversity, Carretera Irapuato-Leon, Mexico: Control of gamete formation by a small RNA pathway in *Arabidopsis.*

SESSION 5: The Maize Genome and General Discussion
Chairperson: S. Tingey, Dupont Experimental Station, Delaware

D. Ware Laboratory, Cold Spring Harbor Laboratory
J. Stein: Genome fractionation/evoluntionary analysis.
J.-M. Chia: Diversity/HapMap.
C. Liang: Gene building and incorporation of RNA-Seq data.



J. Paszkowski, J. Rafalski

D. Ware, M. Timmermans

Psychiatric Genetics: Current Progress and Future Directions

September 23–24

FUNDED BY **The Stanley Research Foundation**

ARRANGED BY **J.D. Watson,** Cold Spring Harbor Laboratory
S.C. Gary, Cold Spring Harbor Laboratory
E. Scolnick, Broad Institute, Massachusetts Institute of Technology

Ted and Veda Stanley and the Stanley Medical Research Institute are most generous supporters of research on schizophrenia and bipolar disorder. Among their initiatives are the Stanley Center for Psychiatric Research at the Broad Institute, and the Stanley Institute for Cognitive Genomics at Cold Spring Harbor Laboratory (CSHL). Both groups are using the latest genome technologies to search for genetic alterations that contribute to schizophrenia and bipolar disorder. This meeting brought Stanley-funded investigators from the Broad Institute and CSHL together, with the goals of providing an update on the research and reviewing topics that may be the basis for future projects and collaborations.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introductory Remarks: **J.D. Watson,** Cold Spring Harbor Laboratory

SESSION 1: Genetics Update
Chairperson: T. Schulze, National Institutes of Health/NIMH, Bethesda, Maryland

J. Sebat, Cold Spring Harbor Laboratory
W.R. McCombie, Cold Spring Harbor Laboratory
P. Sklar, Broad Institute, MIT, Harvard University, Boston, Massachusetts
N. Craddock, Cardiff University School of Medicine, Cardiff, United Kingdom

Coffee break

SESSION 2: Genetics: Next Steps
Chairperson: P. Sklar, Broad Institute, MIT, Harvard University, Boston, Massachusetts

E. Leibenluft, National Institutes of Health/NIMH, Bethesda, Maryland
T. Schulze, National Institutes of Health/NIMH, Bethesda, Maryland

SESSION 3: Neuroscience
Chairperson: L. Tsai, Massachusetts Institute of Technology, Cambridge

K. Singh, Broad Institute, Boston, Massachusetts
A. Sawa, Johns Hopkins School of Medicine, Baltimore, Maryland
P. Osten, Cold Spring Harbor Laboratory
B. Li, Cold Spring Harbor Laboratory
T. Petryshen, Broad Institute, MIT, Boston, Massachusetts

SESSION 4: iPS
Chairperson: A. Malhotra, The Zucker Hillside Hospital, Glen Oaks, New York

H. Song, Johns Hopkins University School of Medicine, Baltimore, Maryland
J. Madison, Broad Institute, MIT, Boston, Massachusetts
K. Brennand, The Salk Institute, La Jolla, California

SESSION 5: Drug Discovery
Chairperson: M. Moyer, Broad Institute, Cambridge, Massachusetts

S. Haggarty, Broad Institute, Harvard University, Boston, Massachusetts
J. Pan, Broad Institute, MIT, Boston, Massachusetts

SESSION 6: Stanley Medical Research Institute
Chairperson: B. Yolken, Johns Hopkins University, Baltimore, Maryland

S. Kim, Stanley Medical Research Institute
S. Sabunciyan, Stanley Medical Research Institute
L. Brando, Stanley Medical Research Institute

SESSION 7: Future Directions
Chairperson: E. Scolnick, Broad Institute, MIT, Cambridge, Massachusetts

Meeting Summary and Discusssion on Future Directions

SESSION 8: Special Session
Introduction: J.D. Watson, Cold Spring Harbor Laboratory

N. Craddock, Cardiff University School of Medicine, Cardiff, United Kingdom
R. Perlis, Broad Institute, Massachusetts General Hospital, Boston
E. Scolnick, Broad Institute, MIT, Cambridge, Massachusetts, and J.D. Watson, Cold Spring Harbor Laboratory

Science: Get It Across!

October 1–8

FUNDED BY **Boehringer Ingelheim Fonds Foundation for Basic Research in Medicine**

ARRANGED BY **C. Walther,** Boehringer Ingelheim Fonds
S. Schedler, Boehringer Ingelheim Fonds

The Boehringer Ingelheim Fonds Foundation returned to the Banbury Center for their biannual fellows meeting in North America. In addition to providing training for their fellows, the Foundation very generously supported a special lecture by a visiting young scientist, given in Grace Auditorium and open to all CSHL scientists. This year's lecture, "Measuring evolution through space and time" was given by Beth Shapiro, Shaffer Assistant Professor from the Department of Biology at Pennsylvania State University.

Opening Remarks: **C. Walther,** Boehringer Ingelheim Fonds, Heidesheim, Germany

Speakers

W. Wells, Global Alliance for TB Drug Development, New York: Writing techniques and how to structure papers.
W. Tansey, Cold Spring Harbor Laboratory: Presentation of graphic information and how to prepare and deliver a scientific talk.
W. Tansey, Cold Spring Harbor Laboratory: Powerpoint presentations and review of videotaped presentations. Writing assignments. Review and critique of videotaped presentations.
H. Ploegh, Whitehead Institute, Cambridge, Massachusetts: What makes success in science?
C. Walther, Boehringer Ingelheim Fonds, Heidesheim, Germany: All about Boehringer Ingelheim Fonds.
B. Shapiro, Pennsylvania State University, University Park: Measuring evolution through space and time at Grace Auditorium.
M. Hansen and M. Corral, Nature Publishing Group, New York: Graphic assignments and presentations.

Aquatic Plants: Environment, Energy, and Evolution

October 18–21

FUNDED BY **The Gordon and Betty Moore Foundation**

ARRANGED BY **R. Martienssen,** Cold Spring Harbor Laboratory
J. Shanklin, Brookhaven National Laboratories
T. Michael, Rutgers University

The aquatic plant duckweed (*Lemna* spp.) has been proposed as an aquatic plant "model" system. They are easy to culture and some species have relatively compact genomes. They propagate vegetatively as well as sexually, have the fastest known doubling time of flowering plants, and are amenable for transient transformation. The Joint Genome Institute recently embarked on sequencing the *Spirodela polyrhiza* genome. Participants in this workshop critically reviewed the biological and genetic properties of duckweed, which may lead to its use in many fields, including plant biology, aquatic biology, biomass, biofuels, metabolic engineering, and bioremediation.

Introductory and Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introductory Remarks: **R. Martienssen,** Cold Spring Harbor Laboratory

SESSION 1: Genetics and Genomics
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

I. Sussex, Yale University, New Haven, Connecticut, and E. Landolt, ETH, Zurich, Switzerland: Comments to the morphological systematic and to the genotypic variability of *Lemnaceae* (read by M. Eberius, LemnaTec GmbH, Würselen, Germany).

M. Edelman, Weizmann Institute of Science, Rehovot, Israel: Lemnaceae: Model plants for in vivo studies.

R. McCombie, Cold Spring Harbor Laboratory: Sequencing *Lemna* and other plant genomes using next-generation sequencing.

T. Michael, Rutgers University, Piscataway, New Jersey: The Duckweed Genome Project.

SESSION 2: Biofuels, Bioremediation, and Other Opportunities
Chairperson: J. Shanklin, Brookhaven National Laboratory, Upton, New York

A. Stomp, North Carolina State University, Raleigh: The Lemnaceae: Tantalizing opportunities and significant challenges.
R. Kerstetter, Rutgers University, Piscataway, New Jersey: The remarkable potential of duckweed as a biofuel feed stock.
C. Xu, Brookhaven National Laboratory, Upton, New York: Regulatory mechanisms controlling biosyntheses and storage in plants and microalgae.
K. Appenroth, University of Jena, Jena, Germany: The affair between duckweeds and heavy metals.
J. Cheng, North Carolina State University, Raleigh: Growing duckweed for nutrient recovery from wastewater.

SESSION 3: Evolution, Ecology, and Education
Chairperson: J. Messing, Rutgers University, Piscataway, New Jersey

C. De Pamphilis, Pennsylvania State University, University Park: Tribe analyses, ancestral genes, and ancient polyploidy.
D. Les, University of Connecticut, Storrs: Phylogenetics and genome evaluation in duckweeds and other aquatic plants.
V. de Miranda, Universidade de Mogi das Cruzes: Aspects of biology, ecology, and evolution of Utricularia (Lentibulariaceae).
B. Greenburg, University of Waterloo, Waterloo, Canada: Use of *Lemna* in environmental toxicology: An ideal system to study mechanisms of phytotoxicology.
T. Oyama, Kyoto University, Kyoto, Japan: Genetic manipulation of *Lemna* in the study of circadian rhythm.
D. Micklos, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Discussion.

SESSION 4: Physiology and Biochemistry
Chairperson: T. Michael, Rutgers University, Piscataway, New Jersey

S. Tresch, BASF Agricultural Center, Limburgerhof, Germany: *Lemna paucicostata:* A plant test organism in herbicide mode of action research.
E. Lam, Rutgers University, New Brunswick, New Jersey: Engineering of duckweed: Plastid transformation.
O. Babourina, University of Western Australia, Perth: Ion transport in aquatic plants.
J. Schwender, Brookhaven National Laboratory, Upton, New York: Duckweeds and metabolic studies using stable isotope tracers.
J. Slovin, U.S. Department of Agriculture, Beltsville, Maryland: Lemnacea as ideal organisms for metabolic pathway research.
J. Cohen, University of Minnesota, St. Paul: Discussion.

SESSION 5: Aquatic Plants and Other Model Systems
Chairperson: T. Michael, Rutgers University, Piscataway, New Jersey

V. Citovsky, SUNY, Stony Brook, New York: Discussion.
T. Mockler, Oregon State University, Corvallis: Application of next-generation sequencing to transcriptome annotation.
M. Eberius, LemnaTec GmbH, Würselen, Germany: High-throughput screening for phenotype and growth to identify and characterize genotype phenotype relation of duckweed.
R. Martienssen, Cold Spring Harbor Laboratory: Developmental challenges and genetic solutions in biofuel crop design.

SESSION 6: Strategies for Future Developments

Discussion moderated by
R. Martienssen, Cold Spring Harbor Laboratory
J. Shanklin, Brookhaven National Laboratories, Upton, New York
T. Michael, Rutgers University, Piscataway, New Jersey

A. Stomp

Feedback Networks in the Intersection of Metabolism and Receptor Tyrosine Kinase Signaling

November 8–10

FUNDED BY **OSI Pharmaceuticals, Inc.**

ARRANGED BY **J. Haley,** OSI Pharmaceuticals

In recent years, it has become clear that proliferative and survival signaling pathways can be made redundant by compensatory signaling through alternative pathways. Many of receptor tyrosine kinase networks are subject to dynamic feedback controls through other pathways that impact the efficacy of single-agent targeted therapies. Similarly, many of the pathways specifically targeted by recent cancer therapeutics are intersected by metabolic control networks that can dynamically alter pathway inhibition and efficacy. This discussion meeting reviewed what is known of signaling networks affecting feedback control of energy utilization, receptor tyrosine kinase signaling, and their intersection nodes and the modeling and imaging of networks and nodes. The goal was to develop a better understanding of how combinations of targeted antitumor agents can overcome the compensatory and feedback control that limits their use as single therapies.

Welcome and Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Background to Meeting: **J. Haley,** OSI Pharmaceuticals, Farmingdale, New York

SESSION 1: Metabolism-PI3K-Tor
Chairperson: J. Haley, OSI Pharmaceuticals, Farmingdale, New York

C. Thompson, University of Pennsylvania, Philadelphia: Metabolic intermediates amplify phosphotyrosine signaling.
M. Simon, University of Pennsylvania, Philadelphia: Oxygen availability and tumor cell metabolism.
L. Cantley, Harvard Medical Center, Boston, Massachusetts: PI3 kinase and cancer metabolism.
D. Sabatini, Whitehead Institute, Massachusetts Institute of Technology, Cambridge: mTOR and the control of cell growth.
P. Dennis, National Cancer Institute, Bethesda, Maryland: Tissue-specific modulation of cancer signaling by metformin in a model of chemoprevention.
W. Weiss, University of California, San Francisco: Akt and autophagy cooperate to promote therapeutic resistance in glioma.
N. Rosen, Memorial Sloan-Kettering Cancer Center, New York: Octogene-induced feedback: Implications for therapy.

SESSION 2: Regulation of RTK Signaling Systems
Chairperson: C. Thompson, University of Pennsylvania, Philadelphia

Y. Yarden, Weizmann Institute of Science, Rehovot, Israel: The ErbB/Her network.
N. Hynes, Friedrich Miescher Institute, Basel, Switzerland: Targeting RTKs in breast cancer: ErbB, Ret, and FGFRs.
P. Carmeliet, Katholieke Universiteit, Leuven, Belgium: Angiogenic strategies by targeting RTK or metabolism.

SESSION 3: Metabolic Controls in the Ras-Raf-Erk and Jnk/p38 Pathways
Chairperson: C. Thompson, University of Pennsylvania, Philadelphia

R. Marais, Institute of Cancer Research, London, England: BRAF therapeutic opportunities.
S. Benkovic, Pennsylvania State University, University Park: De novo purine biosyntheses: The "purinosome."
R. Davis, University of Massachusetts Medical School/HHMI, Worcester: Stress-activated MAP kinase regulation of insulin signaling.

SESSION 4: Network Control of and by Protein Phosphatases
Chairperson: L. Cantley, Harvard Medical Center, Boston, Massachusetts

J. den Hertog, Hubrecht Institute, Utrecht, The Netherlands: Protein tyrosine phosphatases in development and disease.
S. Keyse, University of Dundee, Dundee, Scotland: Regulation of MAPK signaling by protein phosphatases.
T. Tiganis, Monash University, Victoria, Australia: Reactive oxygen species, protein tyrosine phosphatases, and type 2 diabetes.
N. Tonks, Cold Spring Harbor Laboratory: Redox regulation of PTP1B: Novel avenues for therapeutic intervention in diabetes and obesity.

SESSION 5: Global Approaches to Interrogating Networks and Systems
Chairperson: N. Tonks, Cold Spring Harbor Laboratory

M. Comb, Cell Signaling Technology, Beverly, Massachusetts: A new view on Akt signaling downstream from RTKs.
K. Janes, University of Virginia, Charlottesville: Identifying feedback networks in single cells.
W. Hahn, Harvard Medical Center, Boston, Massachusetts: Genetic approaches to dissect feedback and signaling in cancer.

R. Davis

Structural Variation in the Human Genome

November 15–18

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Lupski,** Baylor College of Medicine
E. Eichler, University of Washington

Chromosomal rearrangements were the first genome-scale variations to be discovered in human beings. They were detected by light microscopy of chromosomes stained with simple dyes and later at higher resolution when Giemsa banding was introduced. Fifty years later, extraordinary resolution is being provided by genomic microarrays and sequencing, and new classes of genomic variations are being revealed. Copy-number variants (CNVs) are, perhaps, the most important of these. Ranging in size from kilobases to megabases, CNVs have been associated with a number of human developmental disorders. However, much remains to be done before CNVs can be used routinely as diagnostic markers. The goals of this meeting were to review the occurrence and generation of CNVs in the human genome, discuss approaches for their discovery, examine their relationship with disease, discuss their use for genetic diagnoses, and explore how to develop cost-effective tests.

Introductory and Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introductory Remarks: **E. Eichler,** University of Washington, Seattle
J. Lupski, Baylor College of Medicine, Houston, Texas

SESSION 1: CNVs: The Clinical Perspective
Chairperson: J. Lupski, Baylor College of Medicine, Houston, Texas

B. Bejjani, Signature Genomics Laboratories, Spokane, Washington: Managing CNVs in the diagnostic laboratory.
P. Stankiewicz, Baylor College of Medicine, Houston, Texas: Design of clinical arrays.
H. Firth, Addenbrooke's Hospital, Cambridge, England: Decipher/deciphering developmental disorders.
D. Ledbetter, Emory University School of Medicine, Atlanta, Georgia: CNV atlas of human development.

SESSION 2: Mechanism and Evolution
Chairperson: M. Snyder, Stanford University School of Medicine, Stanford, California

J. Lupski, Baylor College of Medicine, Houston, Texas: Genomic disorders: Mechanisms upstream and downstream from CNV.
E. Hollox, University of Leicester, Leicester, England: Evolution and variation of β-defensin copy number.
J. Sikela, University of Colorado, Aurora: Linking genome instability, evolutionary adaptation, and disease.
P. Hastings, Baylor College of Medicine, Houston, Texas: Mechanisms of structural change.

SESSION 3: Next-generation Detection and Interpretation
Chairperson: E. Eichler, University of Washington, Seattle

M. Gerstein, Yale University, New Haven, Connecticut: Detection and analysis of structural variants in personal genome sequencing data.
J. Korbel, EMBL Heidelberg, Heidelberg, Germany: Next-generation analysis of structural variation in humans with a breakpoint library.
H. Peckham, Life Technologies, Beverly, Massachusetts: CNV detection with short-read sequencing.
M. Snyder, Stanford University School of Medicine, Stanford, California: Variation and transcription factor binding in humans.
E. Klopocki, Charité Universitätsmedizin Berlin, Berlin, Germany: CNVs of conserved noncoding sequence elements: A novel mechanism in the pathogenesis of congenital malformations.
X. Zhang, Chinese Academy of Medical Sciences, Beijing, China: Variable phenotypes associated with CNVs of the same chromosomal regions.

SESSION 4: Neuropsychiatric Traits and CNV
Chairperson: H. Mefford, University of Washington, Seattle

S. Scherer, The Hospital for Sick Children, Toronto, Canada: CNV in autism spectrum and related neurodevelopmental disorders: Genome to outcome.
E. Eichler, University of Washington, Seattle: Genome structural variation and disease.
A. Beaudet, Baylor College of Medicine, Houston, Texas: Genotype/phenotype correlations for CHRNA7 to antisocial behaviors.
J. Sebat, Cold Spring Harbor Laboratory: Looking for rare variants of large effect in schizophrenia and bipolar disorder.

SESSION 5: CNV and Common Complex Traits
Chairperson: A. Beaudet, Baylor College of Medicine, Houston, Texas

H. Mefford, University of Washington, Seattle: CNVs in epilepsy: Expanding the phenotypic spectrum of genomic disorders.
T. Aitman, Imperial College, London, England: CNV and autoimmunity.
S. McCarroll, Harvard Medical School, Boston, Massachusetts: Genome structural polymorphism in common diseases.

SESSION 6: Interpretation of Phenotypic Consequences of CNV
Chairperson: A. Beaudet, Baylor College of Medicine, Houston, Texas

L. Pérez Jurado, University of Washington, Seattle: The hidden variation: Common CNVs/PSVs in autism spectrum disorders.
B. de Vries, University Medical Center Nijmegen, Nijmegan, The Netherlands: Interpretation of CNVs in a clinical setting; benign versus pathogenic.
N. Spinner, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania: Genomic alterations and phenotype: Determining pathogenicity is NOT SO SIMPLE.

Concluding Remarks: J. Lupski, Baylor College of Medicine, Houston, Texas
E. Eichler, University of Washington

Promoting Research on Severe Mental Illness

December 3–5

FUNDED BY **NARSAD, The Brain & Behavior Research Fund, World Heritage Foundation–Prechter Family Fund, Simons Foundation**

ARRANGED BY **J.D. Watson,** Cold Spring Harbor Laboratory
E. Scolnick, Broad Institute
H. Pardes, New York Presbyterian Hospital
H. Heimer, Schizophrenia Research Forum
A. Moran, NARSAD
J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Mental disorders place a terrible burden on society, and there is an urgent need to encourage Congress to promote research on these disorders. A major effort to find the genes involved in mental disorders began in the 1980s, but the then available tools were not suitable for the analysis of disorders caused by many mutations. Continuing technical developments, many arising from the Human Genome Project, have revitalized genetic analysis of complex disorders, and these new techniques are being applied to mental disorders. Participants in this meeting included eminent scientists and psychiatrists, as well as individuals familiar with promoting research, in this case, genomics-based research, an approach offering the best path to treatments or prevention of mental disorders.

Welcome and Introductory Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Welcome and Introductory Remarks: **C. Lieber** and **S. Lieber,** NARSAD, Great Neck, New York
The Need for Research: **J.D. Watson,** Cold Spring Harbor Laboratory

SESSION 1: Genetics and Genomics
Chairperson: E. Kandel, Columbia University, New York

K. Kendler, Virginia Commonwealth University, Richmond: Challenges in genetics research in mental illness.
E. Scolnick and P. Sklar, Broad Institute, Cambridge, Massachusetts: Genomic approaches to mental illness.
W. McCombie, Cold Spring Harbor Laboratory: Fourth-generation DNA sequencing and human genetic disorders.
D. Goldstein, Duke University, North Carolina: Rare and common genetic variants contributing to risk of schizophrenia.
M. Daly, Harvard University, Cambridge, Massachusetts: General discussion.

SESSION 2: Promoting Genomic Research in Mental Illness
Chairperson: T. Insel, National Institutes of Health/NIMH, Bethesda, Maryland

Views of Genetic and Genomic Research
T. Lehner, National Institute for Mental Health
E. Green, National Human Genome Research Institute, Bethesda, Maryland
A. Malhotra, The Zucker Hillside Hospital, Glen Oaks, New York
C. Gilliam, University of Chicago, Chicago, Illinois
F. Henn, Brookhaven National Laboratory, New York
R. DePaulo, Johns Hopkins University School of Medicine, Baltimore, Maryland
H. Akil, University of Michigan, Ann Arbor
W. Bunney, University of California, Irvine

Input from Groups Engaged in Promoting Research
NARSAD: H. Pardes, New York Presbyterian Hospital, New York
Prechter Fund: W. Prechter, Heinz C. Prechter Bipolar Research Fund, Ann Arbor, Michigan
Simons Foundation: G. Fischbach, Simons Foundation, New York

General Discussion and Points Arising

A. Moran, B. Shobe

SESSION 3: What to Do?
Chairperson: H. Pardes, New York Presbyterian Hospital, New York

M. Woolley, Research!America, Alexandria, Virginia: Promoting research.

Is Washington D.C. Receptive to This Message?
G. Weiblinger, National Institutes of Health/NIMH, Bethesda, Maryland: Determining the NIH Annual Budget.

How to Do It?
M.-C. King, University of Washington, Seattle: Scientists joining the advocacy for mental health research.
B. Metheny, South Dartmouth, Massachusetts: Delegation model.
J. Greden, University of Michigan, Ann Arbor: National networks of depression centers.
B. Gill, The Charles A. Dana Foundation, New York: Dana Alliance for Brain Research.

E. Green, J.D. Watson

What Do We Need? Summation and Action Points: Open Discussion
Moderator: H. Pardes, New York Presbyterian Hospital, New York

Coinfections in Lyme Disease

December 14–15

FUNDED BY **Time for Lyme, Inc.**

ARRANGED BY **S. Schutzer,** UMDNJ–New Jersey Medical School

Since the 1990s, the Banbury Center has been the venue for a series of very influential meetings on Lyme disease. A benefit of such meetings has been collaborations into research on coinfections carried by the same tick vector that transmits Lyme disease. This meeting reviewed what is known of coinfections in Lyme disease and provided an opportunity for planning a Lyme disease meeting at Banbury in 2010.

Introductory and Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory
Introductory Remarks: **S. Schutzer,** UMDNJ–New Jersey Medical School, Newark

SESSION 1
Chairperson: S. Schutzer, UMDNJ–New Jersey Medical School, Newark

D. Fish, Yale University, New Haven, Connecticut: Ticks and the environment.

M. Eshoo, IBIS Biosciences, Inc., Carlsbad, California: Summary of polymicrobial detection studies in tick-borne diseases.

SESSION 2
Chairperson: P. Fox, Animal Medical Center, New York

A. Hohenhaus, Animal Medical Center, New York: The dog as a sentinel of tick-borne diseases.

S. Schutzer, UMDNJ–New Jersey Medical School, Newark: Continued and future studies and discussion.

DOLAN DNA LEARNING CENTER

DOLAN DNA LEARNING CENTER EXECUTIVE DIRECTOR'S REPORT

Preparing students and families to thrive in the gene age

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Susan Lauter
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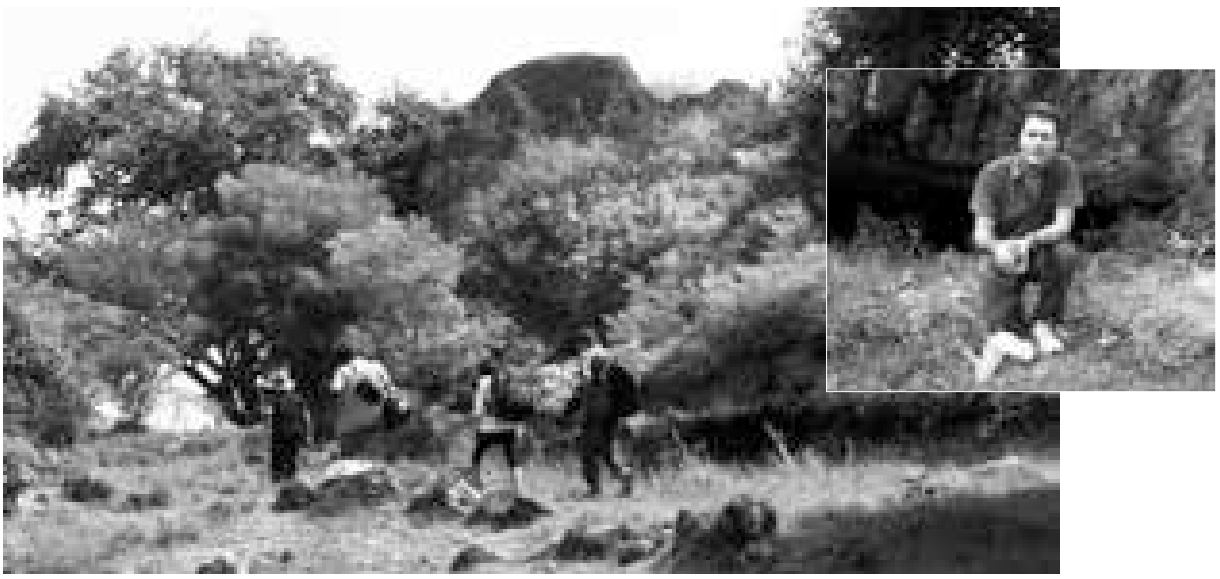
Chun-hua Yang

On November 20, research teams from the United States and Mexico published the complete maize genome—the entire set of genetic information belonging to the plant better known to us as corn. Anticipating this event for the launch of a new Internet site, *Weed to Wonder*, a videography team from the DNALC went to Mexico to get the story behind the research and put the genome sequence into the context of the history of maize cultivation. First, we traveled 70 miles south of Mexico City to the Balsas River Valley, where an old man led us up a shock-breaking road and along an obscure path to a rock shelter from the dawn of maize cultivation. Eight months previously, microscopic analysis of grinding stones from this site revealed traces of cultivated maize that date back 8700 years. This is almost as old as the earliest evidence of wheat cultivation in the Fertile Crescent. The grinding stones found in the shelter were nothing fancy—just rounded river stones—but they would have been effective enough at making a rough corn meal. Combined with water, this would have made a simple porridge or a dough from which to fashion the quintessential Mexican staple, the tortilla.



An elderly guide leads Jason Williams, Eun-sook Jeong, and Dave Micklos—with cameras and tripods in tow—to the site of the shelter where maize grinding stones were discovered. Dave then speaks on camera about the location.

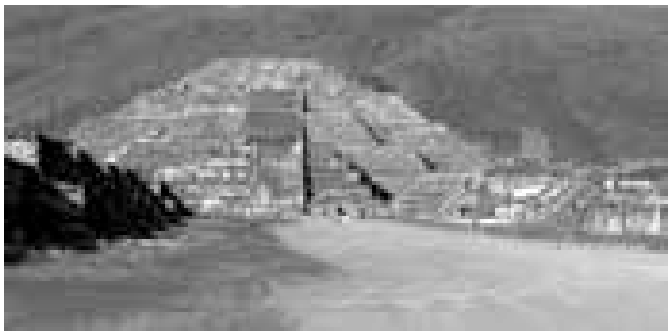
Just below the shelter, we passed a tiny, unkempt corn field very much like the field, or perhaps even the same field, from which the ancient agronomists had harvested their maize. Somewhere nearby, teosinte, the wild ancestor of modern maize, would have been brought under cultivation. Genetic analysis has placed the Balsas River subspecies of teosinte at the root of the "tree" leading to all modern maize. However, the food potential of teosinte would not have been immediately obvious, because its kernels are covered with a flinty, inedible outer covering. In the 1930s, Nobel laureate George Beadle demonstrated how Mesoamericans could have first exploited teosinte as food. He showed that its kernels explode when heated, casting off their flinty coats and leaving behind edible little puffballs. Teosinte was almost certainly eaten as popcorn, making it the world's first and most enduring junk food.

The ancient farmers would have identified stands of teosinte that made the biggest and best-tasting popcorn. Eventually, they would have had the idea to plant seeds from some of the best plants in fertile soil on the banks of tributaries of the Balsas. In watching the cultivated teosinte year after year, they would have selected odd plants—less bushy ones that made harvest easier and, of course, those with larger cobs and less-flinty kernels. For some time, they would have continued to eat the cultivated maize as popcorn, but eventually, they would have discovered the varied uses of ground maize meal. This is the evidence provided by millstones from the humble shelter in the Balsas River Valley.

At the International Maize and Wheat Improvement Center (CIMMYT), we visited Suketoshi Taba, who leads the effort to conserve as many types of cultivated and wild corn as possible. The cold storage vaults of his germplasm bank contain 26,000 varieties of maize seed, the products of thousands of years of human-directed evolution. Represented in this germplasm bank are ~60 indigenous races of Mexican maize—each as genetically distinct from one another as humans are from chimpanzees. Frequent exchange of genes with wild teosinte, which grows as a "weed" in cultivated maize fields, has been a major source of this fantastic genetic diversity.

In Irapuato, 200 miles northwest of Mexico City, we visited the National Laboratory of Genomics for Biodiversity, an immaculately modern facility designed to take Mexico into the genome-sequencing club. There, we interviewed Luis Herrara-Estrella, its director, and Jean-Philippe Vielle-Calzada, who headed their effort to sequence the genome of the maize race called Palomero. They used so-called next-generation sequencing to rapidly generate data on millions of short genome pieces. Then, they "scaffolded" their fragmentary data onto a genome completed by traditional methods by a U.S. team that included CSHL researchers. Vielle-

(*Above*) Teosinte seed, (*right*) Suketoshi Taba discusses one example of maize seed stored in the germplasm bank.




Pyramid of the Moon, Teotihuacan (*left*). Eun-sook Jeong, Jason Williams, Dave Micklos, and Jaime Padilla (the guide for the trip) pose in front of Nevado de Toluca.

Calzada, who spent his postdoctoral period at CSHL, explained that the sequencing of the Palomero genome was simplified because it is ~20% smaller than the B73 cultivar sequenced by the American team. Palomero, with its miniature cob, is one of most ancient of Mexican maize races cultivated as popcorn. A comparison by the Mexican team turned up long stretches of identical DNA sequences shared by the Palomero and B73 genomes. Contrary to the overall sequence differences that arose as maize diversified into different races, these regions must have been strongly selected early in the domestication process and then passed on to all maize races. Close examination revealed that several of these regions contain genes that detoxify heavy metals from the environment, such as cadmium and copper.

With this knowledge, we set off on a jarring 15-mile trip on an unsurfaced road and then a lung-aching hike that took us to pass at 14,000 feet on Nevado de Toluca, a volcanic caldera southwest of Mexico City. One of its Aztec names, *Lord of the Cornstalks*, is appropriate to this story because it looks down on the fields of the Balsas River and is only ~50 miles away from the shelter where we started our exploration. The Toluca volcano erupted most recently ~10,500 years ago, carpeting the region with ash and debris. This coincides closely with the early cultivation of maize and provides a context for interpreting the conserved metal decontamination genes found by Vielle-Calzada. Prior to industrialization, volcanic eruptions were the major source of heavy metals in the soil, and it appears that the cultivation of maize selected for plants that were tolerant to the metallic soils in this region.

Maize gods and goddesses are central icons of every pre-Columbian culture of Mexico, including Mayan, Toltec, Zapotec, Mixtec, and Aztec. There was good reason for this veneration and for the widespread notion that they were "children of the corn." The large-scale cultivation of maize made possible the numerous and vast pre-Columbian cities and the cultures that flourished around them. North of Mexico City, Teotihuacan was a city of 100,000 residents that rose to greatness 2000 years ago. Ears of Palomero maize have been found in graves there. To the east, Cacaxtla was a ceremonial center founded by Mayan settlers. Set atop a towering pyramid, archeologists are still busy excavating one of the most extensive troves of polychrome murals in the ancient world. There, we saw a mural that is perhaps most emblematic of the Native American view of maize and human life, a corn plant in which human heads burst forth from ears of corn along the axis of the stalk. In Mexico, the cultivation of maize is so wrapped up in human history that it is difficult to draw a line between the plant and the people who brought it under domestication.

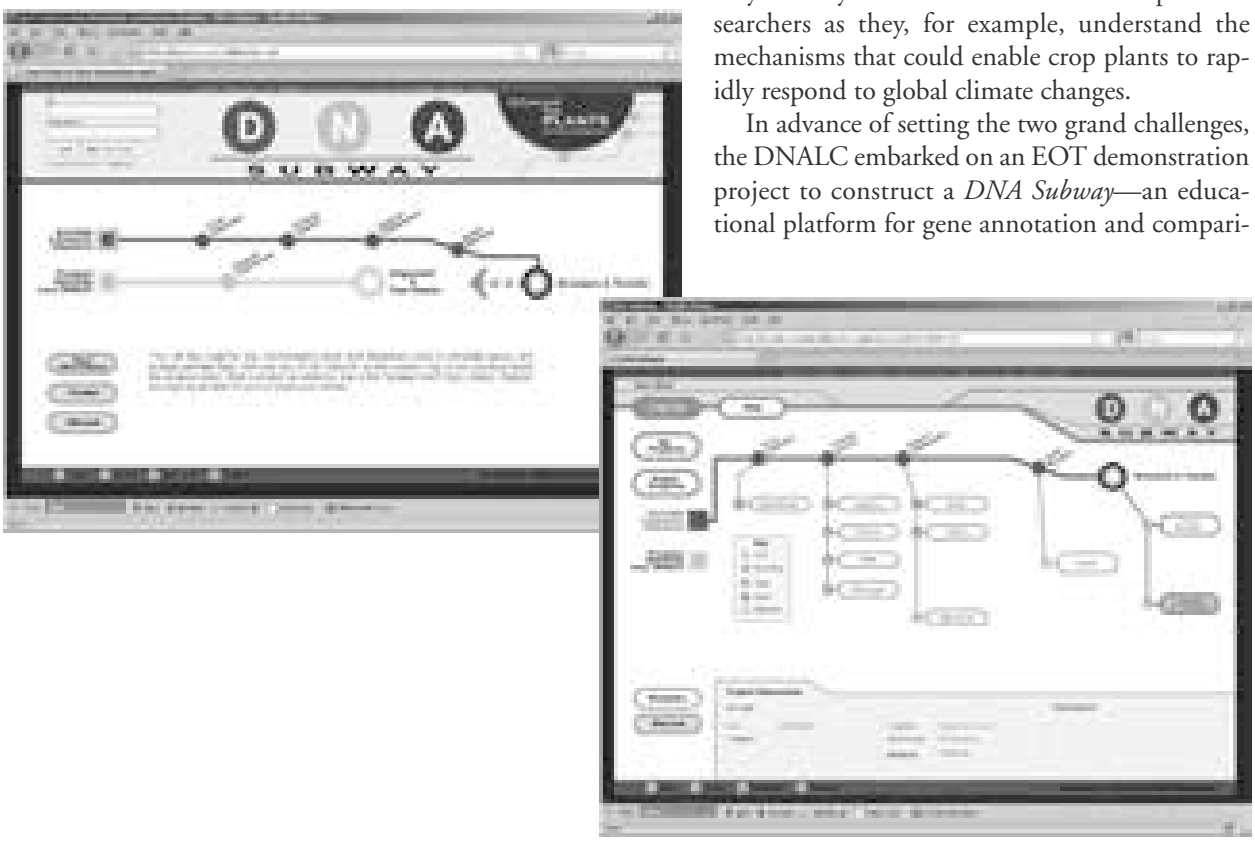
The *Weed to Wonder* Internet site grew out of a collaboration between CSHL's Dick McCombie and Doreen Ware, and Rick Myers of Washington University in St. Louis, to develop a mini-website describing the National Science Foundation (NSF)-funded Maize Genome Sequencing Project. New funding through an NSF project on maize meristem, run by CSHL researcher Marja Timmermans and Mike Scanlon at Cornell University, is allowing us to build a substantial website that explores how humans transformed the common weed, teosinte, into the modern agricultural wonder we know as corn. In addition to a podcast on our Mexican journey, we developed two additional

podcasts with scientists describing CSHL's special connection to the project. Another production was an historical recreation of CSHL in 1909, with George Shull explaining his experiment on hybrid vigor that is the foundation of all hybrid corn grown today. The website includes animations describing the different DNA-sequencing approaches used by the American and Mexican teams and a video tour of the Genome Sequencing Center at Washington University in St. Louis. A virtual exhibit on the life of Nobel Prize–winner Barbara McClintock includes many artifacts from her lab at CSHL, where she discovered transposons (so-called "jumping genes"). An online lab notebook allows students to do experiments in maize genetics and to detect one of McClintock's transposons in corn.

iPlant Collaborative

In 2009 we continued to contribute to the *iPlant* Collaborative (*iPlant*), a consortium headed by the University of Arizona and CSHL to develop a computer (cyber) infrastructure to support plant research. Funded by the NSF's *National Plant Genome Initiative, iPlant* aims to develop tools and interfaces that will provide scientists with easy access to large-scale plant data sets and high-powered informatics tools. Following a year of input from the plant science community, *iPlant* announced in April that it will build "discovery environments" to help plant scientists solve two "grand challenges": (1) to produce an *iPlant* tree of life (*iPTOL*) encompassing ~500,000 plant species and (2) to explore the genotype-to-phenotype continuum in plants (*iPG2P*).

As a member of the *iPlant* Education, Outreach, and Training (EOT) component, the DNALC is working with the grand challenge teams to create educational interfaces for the computing tools and data sets available in each discovery environment. In this way, students and teachers can work with the same data and use the same tools at the same time as high-level plant researchers. Students may 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.



In advance of setting the two grand challenges, the DNALC embarked on an EOT demonstration project to construct a *DNA Subway*—an educational platform for gene annotation and compari-

son. This effort leverages the DNALC's previous experience in developing community workspaces and simple bioinformatics interfaces, such as *Sequence Server* (www.bioservers.org) and *Gene Boy* (www.dnai.org/geneboy/index.html). The project coordinated contributions from 25 scientists, computer programmers, and bioinformaticians at 12 institutions including Scott Cain (Ontario Institute for Cancer Research), Tuajuanda Jordan (Howard Hughes Medical Institute), Zhenyuan Lu and Anthony Biondo (CSHL), Sally Elgin (Washington University in St. Louis), Susan Singer (Carlton College), Ann Stapleton (University of North Carolina, Wilmington), and Sue Wessler (University of Georgia).

We used the metaphor of a subway map as an intuitive and appealing interface to sophisticated informatics tools. "Riding" on any of three different "lines" of the *DNA Subway*, users can analyze up to 100 kb of DNA—predicting and annotating genes (Red Line), prospecting for related genes (Yellow Line), and analyzing next-generation transcriptome data (Blue Line). The first line installed, the Red Line, allows users to (1) predict repeat DNA, genes, and tRNAs, (2) gather supporting RNA and protein evidence, (3) make and edit gene models in a graphical annotation editor (*Apollo*), (4) view the assembled data in stand-alone browser, and (5) export to a community browser to view their assembled data in the context of a completed genome (*Phytozome*). Work can be saved to a personal profile for later use or to share with other users.

In June, we co-organized a meeting at Washington University, St. Louis, to inform our development efforts. The 3-day workshop, *Genomics in Education: Gene Annotation and Comparison*, brought together 44 participants representing three worlds (genome research, education, and computer science) working in three biological systems (microbes, plants, and animals). Presentations emphasized the importance of research partnerships in which students are welcomed as co-investigators. They also highlighted students' desire for a wet-bench hook to anchor abstract bioinformatics investigations. A clear message was that biology educators need to scale up from local experiments that involve a single class to distributed experiments in which classes around the country contribute to and analyze common data sets.

Informed by the consensus developed at the *Genomics in Education* meeting, by year's end, we began organizing two distributed research projects for *iPlant: Chloroplast Gene Sequencing* and the *Orphan Data Project*. The *Chloroplast Gene Sequencing* Project will allow students to analyze sequences to determine phylogenetic relationships between plant groups or to show regional variation within a species (ecotypes). Analyses will be based on so-called DNA "bar codes," short regions of DNA sequence that uniquely identify different plants. Although sequence from the mitochondrial gene *cox1* (cytochrome *c* oxidase subunit 1) is universally used to bar code animals, the plant world has settled on sequences from two chloroplast genes, *rbc*L (ribulose-bisphosphate carboxylase) and *mat*K (maturase K).

Students first work with a plant taxonomist or ecologist to identify interesting local plants whose sequences have not yet been submitted to major bar-code databases. In the classroom, students isolate plant DNA and use polymerase chain reaction (PCR) to amplify the bar-code region from their selected plants. The student DNA samples are shipped to a sequencing center, where the finished sequences are uploaded into a database at the *iPlant* Internet site. Students access their sequences and use them as a starting point in a study of plant phylogenetics or diversity using alignment and tree-drawing tools on the *DNA Subway*. Ultimately, they can submit high-quality sequences to the Bar Code of Life or other scientific databases. This effort will be informed by our existing *DNA Sequencing Service and BioServers* Internet site, which provides sequencing and analysis tools for human mitochondrial DNA.

The *Orphan Data Project* will support distributed research projects in the data-rich world created by next-generation sequencing and high-throughput phenotyping. The objective is to create an online "marketplace" to connect faculty and students with researchers who have novel data sets to be analyzed. The site will provide an index of projects with descriptions of available data and suggestions for how they can be used for class or independent research projects. The site will also include

networking tools—analogous to a Facebook "wall"—to allow collaborators to coalesce around each data set. Partnership Coordinator Jason Williams will facilitate data submissions and "matches" between researchers and interested parties.

Many types of orphan data can be analyzed using the tools available at the *DNA Subway* and *iPlant* discovery environments. Notably, gene mutation and expression data will readily articulate with the genotype-to-phenotype grand challenge. We expect that college faculty will seek out data related to their doctoral or postdoctoral studies, potentially providing projects with additional high-level co-investigators. The Internet is an ideal instrument to identify small communities of interest in a given data set, which would function autonomously from *iPlant*. We expect that some larger communities will reach a threshold at which they might be adopted as formal *iPlant* education projects, and regional workshops will provide venues to share collaborative results.

Landeau Multimedia Studio

In December 2008, *Cable Network News* (*CNN*) eliminated its entire science, technology, and environmental news staff. In March 2009, The *Boston Globe* eliminated its 25-year-old science section. Only 35 of 95 weekly science newspaper sections have survived since their heyday in the late 1980s. In a recent survey of science journalists by *Nature* magazine, 29% said their publications had cut science-writing staff in the past five years. A 2009 survey by the Pew Research Center found that only 20% of adult Americans read science magazines; this is about the same proportion that are considered sufficiently scientifically literate to be able to understand the science section of *The New York Times*. Despite the grim statistics for print science journalism, 67% of respondents in the Pew study said they regularly watch science channels and programs, such as the *Discovery Channel* and *NOVA*. According to Nielsen, the *Discovery Channel* is among the most-watched cable channels.

Somewhere between the waning reach of "hard science" news and the appeal of popular "science lite" lies a niche for informed and entertaining television coverage. With its strong background in Internet multimedia, proximity to CSHL's high-caliber research, and ready access to the flow of 8000 thought leaders in biology who annually attend CSHL meetings, the DNALC is well positioned to occupy this middle ground of television science. We believe that locating a television production team *within* a prestigious scientific community can provide a general model for how science organizations can leverage local expert knowledge to produce high-quality science programming. In this way, universities, research institutes, and science centers can help fill the vacuum created as the mainstream mass media retreat from science coverage.

The completion of the *Laurie J. Landeau Multimedia Studio* has provided us with the physical resources needed for high-level video production. During the year, we demonstrated capabilities of the studio's theatrical lighting and sets by producing video in a number of different formats. In addition to interviews for our *Genes to Cognition Online* and *Weed to Wonder* Internet sites, we completed news podcasts on several topics: an anthropology series on ancient hominid fossils "Ida," "Ardi," and Neanderthal; the Nobel Prize in Physiology or Medicine; and the completion of the maize genome.

We are now working to come up with concepts and funding mechanisms that will enable the DNALC to partner with PBS and cable channels to produce high-quality television programs. Talks with Robert Krulwich of ABC News and WNYC's *Radio Lab*, and Laura Savini, of WNET.org, helped crystallize one concept for an inquiry-based program that shows how DNA

For a *Weed to Wonder* podcast, James Watson (*left*) provides perspective on the role maize has had in CSHL's history.

solves real problems in modern life. Equal parts talk show, cooking show, and investigative report, each episode will take the audience inside an interesting "detective case." Although it will build on popular interest in *Crime Scene Investigations* (*CSI*), the show will explore how modern science can solve a range of interesting, everyday problems. The program will employ a young, "perky-quirky" host who begins each exploration at the studio coffee table brainstorming with CSHL scientists about how to solve a real-world problem. (The host and scientists are "the DNA detectives.") Taking to the field, the host personalizes the problem by interviewing people and gets experimental ideas by visiting the lab of a scientific expert. Then, it's back to the studio lab, to "cook up" a real experiment with the help of the detectives. The experiment will answer the question at hand and also point to solutions for similar problems.

For example, a recent exposé found that many restaurants substitute tilapia and other inexpensive fish for premium fish, such as red snapper, white tuna sushi, and Chilean sea bass. In this case, the DNA detectives would follow several New York families into restaurants and interview them while they order a premium fish. The detectives then take samples back to the studio to run DNA tests to determine exactly what sorts of fish are on the menus. Other questions the DNA detectives might tackle include: Do perfume testers smell better than me? Is my certified organic food genetically modified? Which top chef has the best sense of taste? How can DNA set free a convict on death row? Where did that illegal elephant tusk come from? Is it safe for me to take that drug? Am I related to a Neanderthal? How do they find a good mate for a zoo animal? Is my all-beef hotdog all beef?

Genes to Cognition (G2C) Online

Our latest, largest, and most interactive website, *Genes to Cognition* (*G2C*) *Online* (www.g2conline.org), launched on March 15, coinciding with the beginning of the Dana Foundation's Brain Awareness Week. *G2C Online*, which is supported by the Dana Foundation and Hewlett Foundation, features a rich library of more than 750 animations, articles, demonstrations, and interactive experiments about neuroscience in the 21st century. It is modeled as a small world network, where each content item is a node in a web of interactive media. By exploring nodes at any of six levels of analysis—genes, biochemicals, cells, brain, cognition (behavior), and the environment—students can understand that thinking and disorders of thinking can be approached on multiple levels. Six major psychiatric disorders form the hub of the *G2C Online* network: ADHD, autism, Alzheimer's disease, bipolar disorder depression, and schizophrenia. Students can learn the fundamentals of neuroscience through a series of narrated animations, conduct interactive experiments using real-world experimental data, and browse a library of video interviews with leading researchers, including Nobel laureates Eric Kandel and James Watson.

The most searched-for feature is the *3D Brain*, an interactive brain map that allows users to rotate the brain in three-dimensional space. Created in collaboration with *AXS-3D*, a Toronto-based animation studio, *3D Brain* includes individual maps of 29 substructures with information on brain damage, case studies, and links to modern research. We collaborated with Jens Egeblad, brother of CSHL's Dr. Mikala Egeblad, to create *3D Brain*, the DNALC's first iPhone application. Launched on September 20, the *3D Brain* app has become a staple in *Apple*'s Education Top-10, peaking at some 20,000 weekly downloads. By year's end, it had been downloaded 159,380 times to users in 70 countries and was the highest-rated app in the Education Top-25. *3D Brain* is free to download from *Apple*'s iTunes and the App Store.

The *G2C Online* project will culminate in 2010, with a large-scale study of how the site is used in classroom settings. Twelve high school and college educators and their 700 students will participate in a quasi-experiment, which uses matched experiment and control groups. Each teacher will use the site with two classes they teach. One class will use *G2C Online* to learn about a selected topic (e.g., autism) and use traditional resources to learn about another topic (e.g., schizophrenia). A second class will do the reverse: Use traditional sources to learn about autism and *G2C Online* to learn about schizophrenia. Using classes taught by the same teachers, controls for the level of background and "scaffolding" that accompanies each topic, while "flipping" the assignments controls for class differences. As well as completing teacher-authored assessments, students will also complete a number of instruments designed to assess science literacy and general knowledge of neuroscience. In this way, we hope to find out if *G2C Online*, and its unique network structure, helps students conceptualize "ideas" in science.

Internet Visitation and Development

Although the number of visits to the DNALC's family of Internet sites in 2009 held constant at six million, visitors consumed 23% more information than in 2008. Nearly 5 terabytes (5000 gigabytes, GB) of data were served, with *DNA Interactive* reaching 1365 GB for the year. Two new sites were added in 2009. *Genes to Cognition* (*G2C*) *Online* launched in March, and *Weed to Wonder* launched on November 20 to coincide with the publication of the maize genome sequence in the journal *Science*.

We continued with a series of interventions to update our websites and to drive visitation. This included search engine optimization to make our sites more visible to *Google* and other search arbiters. We devoted considerable effort to redevelop the DNALC home page, which provides information about our educational programs and is a portal to our 14 content sites. The former home page was built in the media integration program *Flash*, which provided a sleek design but hid some content from search engines. The new design uses HTML pages formatted with cascading style sheets (CSS). The HTML pages are readily searched, and CSS enables us to rapidly update the site.

Periodically "freshening" sites with new content encourages visits from search engines indexers, which can improve page rank in search results. Notably, we added blogs to six of our major content sites: *Genes to Cognition Online, Inside Cancer, DNA Interactive, Eugenics Archive, DNA from the Beginning*, and *Your Genes, Your Health*. On a regular basis, DNALC staff members provide their own perspectives on current news and discoveries that connect with website content. Blog visits totaled 32,066 in 2009. We have also broadened DNALC exposure by creating *Twitter* and *Facebook* pages.

We increased access to our high-quality video content under our own *DNA Today* banner and through the *YouTube* channel. We posted 82 videos on *YouTube* over the course of 2009 resulting in nearly 42,000 video views, more than half of the total views (76,213) since we started *YouTube* postings in May 2007. The majority of viewers found our videos by searching *YouTube* (29%) or by following a link from another video page (30%); perhaps surprisingly, only 5% of *YouTube* views were initiated by Google searches. *Cell Signals*, a three-dimensional tour through a cell, comprised 57% of *YouTube* views, followed by The Neanderthal Genome Project, an interview with Svante

	Average visit length	Change from 2008	Average monthly bandwidth (GB)	Change over 2008	Visits in 2009	Change from 2008 (%)
Content sites						
Blogs (G2C 3/09, others 8/09)	10:21	n.a.	1.86	n.a.	32,066	n.a.
DNA from the Beginning	12:49	+3:17	29.74	–7.35	1,180,507	–9.34
DNA Interactive & myDNAi	10:26	0	113.73	+2.17	1,046,656	–9.07
Dolan DNALC Home	10:05	+0:52	134.27	+39.5	1,623,778	–0.34
Genes to Cognition Online (3/15)	8:34	n.a.	44.39	n.a.	186,574	n.a.
Image Archive on the American Eugenics Movement	14:04	–3:42	17.57	+6.02	687,515	+13.08
Inside Cancer	9:48	+2:30	29.30	–2.05	187,311	–21.93
Inside Cancer Teacher Center	10:53	–3:08	0.54	–0.49	86,707	+289.68
Your Genes, Your Health	7:55	–0:17	37.99	–3.46	691,320	–14.19
Weed to Wonder (11/20)	18:46	n.a.	37.55	n.a.	16,277	n.a.
Laboratory/bioinformatics sites						
BioServers	20:11	–3:56	2.57	+ 0.01	100,929	–25.76
DNALC Kits/Carolina Collaboration	9:11	–6:07	0.66	–1.68	8,910	+64.24
Dynamic Gene	9:45	+2:27	0.34		15,514	+37.57
Genetic Origins	6:38	–0:50	1.53	–0.04	102,163	–0.66
Greenomes	3:34	+0:19	0.47	–0.07	17,317	+30.09
Silencing Genomes	11:01	–1:44	0.54	–0.01	38,913	+32.08
All sites	**10:54**	**+1:27**			**6,022,457**	**–0.62**

Pääbo, at 10%. *YouTube* reports that our audience is largely male (62%) and between 45 and 64 years of age (53%), highlighting a need to attract female and younger viewers.

As part of the National Science Digital Library Project, we completed a 4-year project to develop a database of virtually all of the DNALC's proprietary multimedia content. This content management system constitutes a digital card catalog that allows any item to be readily searched using a large set of fields. Indexing 727 items from our first site, *DNA from the Beginning*, brought to 5380 the total number of animations, videos, images, photographs, and other items in our content management system. All of these items were "harvested" to the BioScieEdNet (BEN) digital library, composing more than one-third of the 15,319 resources available at this NSF-supported portal (www.biosciednet.org). The DNALC home page incorporates a new search interface that searches all of these items, an increasing number of which can be downloaded to personal computers.

We continue to get a visitation boost from *Google AdWords*, a grant program that provides free "sponsored" links in the right-hand-side "advertising gutter" on *Google*'s search results page. The *AdWords* account contains a set of keywords for each DNALC website. When someone searches for one of the keywords, an ad for the site is displayed and logged as an "impression." A "click-through" is logged when the link is followed, resulting in a visit to one of our sites. *DNA from the Beginning* is our most successful site on *AdWords*, with 57,254 visits from 3,557,433 impressions. Not surprisingly, 86.6% of click-throughs resulted from a search for "DNA." *Genes to Cognition Online* reaped 55,025 visits from 4,949,401 impressions—73% were searches for "brain." All DNALC sites received a total of 140,201 visits from 13,128,457 *AdWord* impressions. The relatively low conversion rate (from impression to click-through) is probably due to the fact that users of free content, such as teachers and students, generally concentrate on free listing rather than sponsored ads. The rankings of free listings also give a measure of the authority of the link, and DNALC sites are frequently among the top five returns for many key search terms.

Faculty Training

During the year, more than 1200 educators participated in a range of professional development activities conducted at *Harlem DNA Lab* and at 19 sites around the United States and Asia. Through our collaboration with the New York City Department of Education, sponsored by the Howard Hughes Medical Institute, 312 teachers participated in workshops at *Harlem DNA Lab,* including 37% underrepresented minorities. With funding from the NSF, National Institutes of Health (NIH), and the Hewlett Foundation, we collaborated with host institutions to conduct 37 workshops on cancer biology, RNA interference (RNAi), and neurobiology. Seven workshops were conducted at institutions with high proportions of underrepresented minorities, where 20% of participants were African American or Hispanic. Overall, 12% of the 590 participants at off-site workshops were underrepresented minorities. An additional 319 educators attended workshops or presentations at professional meetings, which covered topics including neurobiology, bioinformatics, detecting GM foods by PCR, DNALC online tools for education, and DNA forensics. One workshop was held at Ho Yu College in Hong Kong as part of the inauguration of *MobileLab*, an impressive biotechnology bus developed by our friend William Mak.

With Phase II funding from NSF's Course, Curriculum, and Laboratory Improvement (CCLI) program, we continued our efforts to bring compelling RNAi experiments into college classrooms. A total of 126 college faculty participated in week-long workshops and weekend follow-ups that cover a range of RNAi experiments in *Caenorhabditis elegans*, a simple roundworm that is a model eukaryotic system. After observing mutant phenotypes and learning basic worm "husbandry," faculty learned simple methods to induce RNAi and then learned "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 methods to support student projects, such as using bioinformatics to identify a target gene and developing their own RNAi reagents "from scratch." The *Silencing Genomes* Internet site (www.silencinggenomes.org), which has received more than 80,000 visits, includes all experiments and reagent recipes. A free-strain library includes all needed bacterial and *C. elegans* strains, as well as more than 100 vectors developed by workshop participants to silence worm homologs to human genes. To date, more than 1300 strain orders have been filled and used with a reported 6000 students. Three stand-alone kits derived from the program will be released by Carolina Biological Supply Company in 2010.

With Phase II funding from an NIH Science Education Partnership Award (SEPA), we continued to improve and disseminate *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

Faculty Workshop Sites 2009

Silencing Genomes, Inside Cancer, and G2C Online: Virginia Tech, Blacksburg

Inside Cancer and G2C Online: Arizona State University, Tempe; Contra Costa Office of Education, Pleasant Hill, California; Great Bay Community College, Portsmouth, New Hampshire

Harlem DNA Lab, New York: John Jay College of Criminal Justice, New York; Madison Area Technical College, Madison, Wisconsin; Oxnard College, Oxnard, California; Raritan Valley Community College, Somerville, New Jersey; Tulsa Community College, Tulsa, Oklahoma; University of Colorado, Denver

Silencing Genomes and Inside Cancer: Houston Community College Northwest, Houston, Texas; Howard University, Washington, D.C.; North Carolina A&T University, Greensboro; St. Louis Science Center, Missouri

Silencing Genomes: Austin Community College, Austin, Texas; California State University, Dominguez Hills

Inside Cancer: Bossier Parish Community College, Bossier, Louisiana; Illinois Institute of Technology, Chicago; Minneapolis Community & Technical College, Minneapolis

the science process while engaging students with this relevant topic. A total of 314 high school and college faculty attended 1-day workshops at 18 sites around the nation, learning how to use *Inside Cancer* to enhance teaching of cancer cell biology in health, general biology, and advanced biology classes. Responding to feedback from workshop participants, five Faculty Fellows spent 3 weeks in residence at CSHL creating lessons and improving *Inside Cancer* content: Margaret Witecki (Mount Vernon High School, Washington), Wendy Wooten (High Tech Los Angeles High School, California), Lisa Orenstein (The Overlake School, Redmond, Washington), Robin Cochran-Dirksen (Lead-Deadwood High School, South Dakota), and Greg Ballog (South Whidbey School District, Langley, Washington). With funding from the Hewlett Foundation, we continued to disseminate *G2C Online*. One-day workshops conducted at 11 sites drew 150 high school and college educators to learn how to use this unique learning resource.

We continued our long-term collaboration with the Singapore Ministry of Education when in December we hosted an attachment of four Singaporean primary teachers (grades 3–6). The visiting educators spent 2 weeks in Cold Spring Harbor, immersed in student programs and in small workshop sessions with DNALC middle school instructors. Over the course of their stay, the teachers focused on the Fun with DNA and World of Enzymes curricula and will share what they learned with colleagues in their respective schools.

Harlem DNA Lab

With the official opening of *Harlem DNA Lab* in fall 2008, we fulfilled our long-held goal of developing a base of operations from which to provide underserved schools in New York City (NYC) the same enrichment opportunities that we offer Long Island's schools. The NYC Department of Education renovated the 1200-square-foot facility, located in the John S. Roberts Educational Complex to our specifications and the Jerome L. Greene Foundation provided $100,000 of state-of-the-art equipment.

Core support from the Dana Foundation allowed us to aggressively ramp-up our student enrichment program to reach our target audience of underserved and disadvantaged students. In 2009, we provided lab field trips to 3183 middle and high school students, 78% of whom were African American or Hispanic. Eighty percent of students were provided scholarships courtesy of the William Townsend Porter Foundation, based primarily on Title One status (at least 40% of a school's student population is on free/reduced lunch program). Students came from 45 zoned, charter, magnet, and independent schools from throughout New York City.

We provided intensive enrichment to students from two schools housed with us in the John S. Roberts Educational Complex: the Coalition School for Social Justice and MS 45. Sixty Coalition students (grade 12) participated in a series of three biotechnology laboratories, and 375 MS 45 students (grades 6–8), participated in three genetics laboratories. A Coalition senior was recruited as our first student intern. In the spring, we participated in several programs for the World Science Festival (WSF), "a tribute to imagination, ingenuity and inventiveness that takes science out of the laboratory and into the streets, theaters, museums, and public halls of New York City." For *Pioneers in Science*, we worked intensively with immigrant students from Brooklyn International High School to prepare for one-on-one in-

Following a bacterial transformation, participants "painted" with *E. coli* in collaboration with artist Amy Chase Gulden at the World Science Festival.

terviews with Nobel laureate Harold Varmus during the Festival. At our booth at the *Street Fair* in Washington Square Park, Ileana Rios did DNA extractions with passersby.

In late fall, we announced a *Charter Membership Program* to provide intensive support to several independent schools as they develop a sequenced program of accelerated science opportunities for their teachers and students. An exclusive package of benefits includes in-school and laboratory field trips, summer camp programs, and professional development for teachers, all designed to bring science instruction up to par with the most advanced schools in the country. By year's end, Trinity School, one of the oldest independent schools in Manhattan, had made a 2-year commitment. We expect that the remaining membership will be filled early in 2010.

HHMI Training Program

Harlem DNA Lab is also the site of a professional development collaboration with the NYC Department of Education (DOE). Sponsored by the Howard Hughes Medical Institute (HHMI), the program aims to develop a strong base of teachers who can competently introduce six "targeted" experiments in genetics and biotechnology at identified points in required science courses. We worked intensively with DOE staff members and project advisors to introduce the training program to key opinion leaders in the educational system. During the second year of the project, we provided 612 training sessions (one teacher x 3–4 hours of lab training), fulfilling 77% of our ambitious 800-session goal. Fifty-eight teachers completed four sessions required for Certificate Training, and 378 teachers had taken one or more labs. Almost two thirds of training sessions were within our primary audience of 8th-grade *Scope and Sequence* teachers and 9th–10th-grade *Living Environment* teachers and included 35% African American and Hispanic teachers.

We also continued to work on two key project components that support classroom implementation: online *Lab Center* and experiment footlockers that support each of the targeted labs. *Lab Cen-*

DNALC educators provide video introductions to each *Lab Center* activity. Teachers can use *Lab Center* to prepare students before their visit and for follow-up afterward.

ter (www.dnalc.org/labcenter/harlemdnalab) provides multimedia guides for each lab, including video introduction, online lab notebook, animations, scientist interviews, teacher lesson plans, and prelab and postlab student work sheets. Teacher Fellows from around the New York metropolitan area continued to help the website evolve: Greg Borman (City University of New York), Caren Gough (New York State Science Mentor), Dr. Dahlia McGregor (South Shore High School), Adrienne Rubin (Eleanor Roosevelt School), Kathleen Rucker (Brooklyn International High School), and Jerry Watkins (Central Islip). The final phase will be to add a series of "New York Stories," video podcasts that link each experiment to notable work, past and present, done by researchers in New York area institutions.

Images of student and gel taken by a teacher using a Footlocker kit electrophoresis digital camera.

Our original proposal envisioned regional consortia of individual schools that would contribute funds to purchase shared footlockers. The recession had effectively ended this source of local funds, putting the footlockers in jeopardy. However, a grant of $75,000 from the Richard M. Lounsbery Foundation jumpstarted the program in October with the purchase of 14 footlockers made to our specifications. For a small restocking fee, teachers who have participated in professional development training or who have accompanied students on a field trip to the *Harlem DNA Lab* are invited to rent footlockers. Kits contain all equipment and materials needed for labs on DNA models, fruit fly mutations, DNA extraction, bacterial transformation, protein isolation, DNA restriction analysis, and human DNA variations.

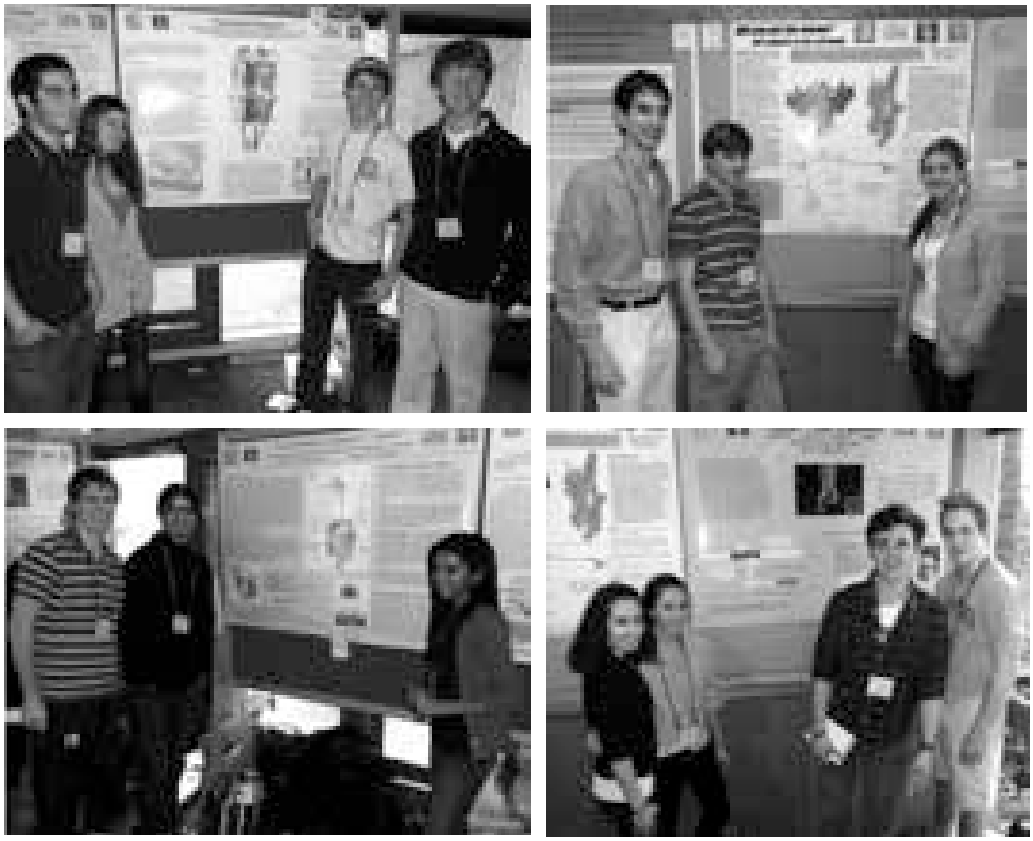
Student Instruction and Family Science

Annual visitation reached 47,798 in 2009. This included 21,031 students who conducted experiments at the DNALC, DNALC West or the *Harlem DNA Lab*, and 9450 students who received in-school instruction by DNALC staff members. About two-thirds of student visitors participated in *Genetics as a Model for Whole Learning* (*GMWL*), a program that is used to introduce genetics at 70 public and private schools. Bank of America and TD Bank provided scholarships for 2032 students from underserved Long Island schools.

Despite difficult economic circumstances, hard work by DNALC administrators and educators made the 2009 summer camps a great success. A record 932 students attended camps at the DNALC, DNALC *West, Harlem DNA Lab*, and in Wyandanch. A new online system simplified registration and saved administrative time by enabling parents to register and pay for camps, switch sessions, and cancel registrations on their own. Frequent ads in local papers, including *Nassau, Suffolk*, and *Big Apple Parent*, and large e-mail campaigns also contributed to the successful summer season. With the help of Jerry Watkins, a retired Central Islip science teacher and long-time friend of the DNALC, 12 students from Wyandanch High School participated in a 1-week DNA Science camp held in the most unlikely of places: the Wyandanch Public Library! Brookhaven National Laboratory provided stipends for all students, and the DNALC provided materials and reagents. In addition, Bank of America and the William Townsend Porter Foundation provided scholarships to 19 underrepresented minority or disadvantaged students who attended camps at the DNALC and DNALC *West*.

We continued the spring tradition, begun in 1985, of hosting *Great Moments in DNA Science*, a seminar series that honors the brightest local students. Three in-depth talks covered the gamut of research at CSHL and drew 271 participants: Dr. Eric Sawey, "Of Mice and Men: Using a Mouse Model to Identify Human Oncogenes"; Dr. Emily Hodges, "Using Massively Parallel Sequencing Technology and Genome Sequencing to Study Cancer Genomes"; and Dr. Stacy DeBlasio, "Illuminating Biology—The Science of Fluorescent-tagged Maize." *Saturday DNA!* continued its popular monthly run, with hands-on sessions for parents and children that feature the latest developments

in the biological sciences. DNALC instructional, scientific, and *BioMedia* staff led 408 participants in programs on diverse explorations, including "Life up Close: Microscopic Worlds," "Personalized Medicine," and "Composting 101."



Cold Spring Harbor High School students pose with their protein posters.

CSH High School Partnership

In the spring, we graduated our third class of students from *Genome Science*, our partnership with Cold Spring Harbor High School (CSHHS). The year-long "capstone" course was co-instructed by DNALC staff and CSHHS biology teacher Martin Glynn, with students coming on alternate days to the DNALC for their final two class periods. The course emphasized critical thinking and included experiments and independent projects across a range of biological systems: A plant unit analyzed transgenes in genetically modified food and newly sequenced genes in rice; human-based experiments used molecular tools to examine human origins and genetic variation; and work with *C. elegans* introduced the cutting-edge RNAi technique.

The fourth class of *Genome Science* commenced in the fall with the added benefit of students coming to the DNALC 5 days a week. The basic units on plants, RNAi, and human variation are being supplemented with new research projects. As advance work on our *Chloroplast Gene Sequencing* Project, the students tested primers and produced a DNA bar code to identify local plants. In collaboration with Tim Herman at the Center for BioMolecular Modeling at the Milwaukee School of Engineering, and Joan Kiely at the Biotechnology Teaching Center at Stony Brook University, we have added a new module on protein modeling. As part of the bioinformatics unit, the students re-

searched proteins involved in cell signaling and used three-dimensional software to highlight important parts of their structures. Coordinates from their modified structures were then used to construct physical models of the proteins. The students presented their models and created posters describing their proteins to scientists at a CSHL meeting on "Rat Genomics and Models." We are also pioneering a new section on metagenomics, which uses high-throughput sequencing to survey genes and organisms present in an ecosystem. In this case, students will use bioinformatics tools to search sequences from microbes in rotting wood, with the aim of identifying novel enzymes that break down plant cellulose into sugars that can be used for biofuel production. This collaboration involves former DNALC instructor Scott Bronson and researchers Niels Van der Lelie and Safiyh Taghavi at Brookhaven National Laboratory.

Watson School of Biological Sciences

Each January, students from the Watson School of Biological Sciences take a required teaching rotation, during which they work at the DNALC to learn effective skills for communicating with nonscientists. In the first phase of training, Watson School students observe DNALC instructors as they teach middle and high school labs. Students then work in pairs to develop lesson plans that will guide their own instruction. In the second phase of training, each graduate pair coteaches their prepared lab alongside a DNALC instructor. Detailed feedback on their teaching—including pace, knowledge, and engagement—prepares each pair for the final training phase: independently teaching the practiced lab (with DNALC staff available for support). After completing middle school and high school rotations, students select three additional lessons to demonstrate their new teaching skills or travel with DNA staff to provide off-site instruction at a local middle school.

At the annual Watson School commencement on April 26, DNALC Executive Director David Micklos was among four people whose contributions to science education were recognized with an honorary Doctor of Science. Accepting the degree, he highlighted the unique opportunity that the DNALC presented to him, to CSHL, and to Long Island:

Laurie Landeau, CSHL Trustee and DNALC Committee Chairperson, introduces Dave Micklos before he receives his degree. Below, Dave Micklos (*left*), David Spector (*center*), and Bruce Stillman (*right*).

> "Jim Watson is fond of saying that one should never be the brightest person in the room. I was acutely aware that this applied to me when I first came to Cold Spring Harbor 26 years ago. Whatever success I have had here was in large part motivated by my desire to fit in amongst the fabulous minds at Cold Spring Harbor, and the honor of this degree makes me feel that I have found a small niche in this great institution. I am very proud of what we have been able to accomplish at the Dolan DNA Learning Center. When I first came to Long Island, most students remembered Cold Spring Harbor for its Fish Hatchery. Now we have a generation of students who remember Cold Spring Harbor for its DNA—and fifth and sixth graders are getting the grounding in

the genetics they will need to flourish in the genome age. I want to make it clear that I really don't believe that the unlikely success of the DNA Learning Center could have been repeated at another institution. The DNA Learning Center arose from unique brew of high-level scientists and engaged philanthropists, reaching out to include young students in the seamless flow of the Laboratory's history and march into the future."

DNA Sequencing Service

The DNALC provided another year of free DNA sequencing to students and teachers worldwide. The *DNA Sequencing Service* allows users to analyze and compare their own mitochondrial (mt) DNA sequence with populations from around the world to explore human diversity. The mitochondrion is the cellular organelle responsible for providing energy in higher cells. A remnant of its ancient life as a free-living bacterium, the mitochondrion has its own chromosome (genome). Depending on energy demands, there are hundreds to thousands of mitochondria per cell, and each carries several copies of mitochondrial chromosome. Thus, mitochondrial DNA is highly amplified compared to nuclear DNA, which has only two copies. This makes mitochondrial DNA the ideal target for sequencing from tissue samples that are very old, very small, or badly degraded by the environment. The hypervariable region of the mitochondrial chromosome accumulates mutations quickly, providing a means to study genetic changes that have occurred during human evolution.

The DNALC popularized methods to use mitochondrial mutation analysis in education, focusing on the same DNA region used by *National Geographic*'s Genographic Project and featured in the popular book *The Seven Daughters of Eve*. Using DNALC protocols or ready-to-use kits, students isolate a 440-nucleotide sequence of their mitochondrial genome and send the amplified samples to the DNALC by overnight mail. Sequencing reactions are prepared by DNALC interns and sent to the CSHL Sequencing Shared Resource Facility in Woodbury for sequencing on an Applied Biosystems 3730xi Genetic Analyzer. The finished sequences are uploaded to an online DNA database at the DNALC's *BioServers* Internet site (www.bioservers.org), which provides tools to compare mitochondrial DNA sequences of individuals across the globe. Additionally, mitochondrial DNA sequences from Neanderthals (~50,000 years old) and Otzi "the ice man" (5000 years old) allow students to consider the question "how ancient is ancient?" In 2009, we sequenced 7057 student DNA samples submitted from 121 high schools, 39 community colleges, and 39 universities. The free sequencing service, which is now in its 11th year, is made possible by sequencing reagents donated by Applied Biosystems of Foster City, California.

Staff and Interns

In the fall, we said goodbye for a second time to Malissa Greif (formerly Hewitt). Malissa helped found and then managed our successful GMWL program for middle schools in the mid 1990s, leaving in 1998 to become a full-time mom. She returned in 2006 as a middle school instructor, recruiting a number of new schools into the GMWL program and developing new labs on bioremediation. We wish her the best of luck in her new home in Nissequogue.

As development of the *Genes to Cognition Online* website wound down, multimedia designer Stephen Blue left to concentrate on his teaching at City University of New York and Parsons School of Design. In addition to his work on *G2C Online*, Stephen, who joined us in 2007, designed the multimedia *Lab Centers* (www.dnalc.org/labcenter/) that accompany six of our most popular lab field trips. During the year, he continued to collaborate with CSHL Press, developing illustrations for our forthcoming textbook, *Genome Science*.

In December, high school instructor Jason Williams was promoted to the newly created position of partnership coordinator for the *iPlant Collaborative*. Jason will have a key role in developing large-

scale, distributed experiments that allow faculty and students to participate directly in modern plant research. He will work with the plant research community to identify available tools and data sets and then foster ongoing collaborations among scientists, teachers, and students. One project will provide online tools and databases to allow students to sequence and analyze genes used to study plant phylogenetics and diversity.

New DNALC instructor Jennifer Aiello.

After graduating *Magna Cum Laude* from Long Island University with a bachelor's degree in forensic science and a minor in chemistry, Jennifer Aiello joined the DNALC staff as a middle school instructor. Jen started here in 2002 as a high school intern from Kings Park High School, continuing to work at the DNALC through her years at C.W. Post, when she managed our free DNA *Sequencing Service* and our *C. elegans* strain bank. Jen has already brought new experiences in forensic science to our *Saturday DNA!* Program, which we anticipate will grow into a new summer camp offering.

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: Laura Bergsten (Cold Spring Harbor), George Economou (Syosset), Lindsay Hochberg (Oyster Bay), Anouva Kalra-Iall (Roslyn), Anastasia Minkin (Oyster Bay), Nate Rahimzada (North Shore Hebrew Academy), Jaylin Sasson (Jericho), Max Vaysman (Commack), Pamela Wax (Harborfields), and Sara Wienclaw (Kellenberg). We also welcomed Annie Laurie Benzie (Adelphi) as a college intern to help with DNA *Sequencing Service* and *C. elegans* strain requests. Returning interns were Emily Troge (Our Lady of Mercy) and Kevin Wu (Jericho). Many of our interns continued to invest their DNALC experience in their own research. Arielle Scardino (City College) worked with Jermel Watkins to explore how a decreased expression of certain mitochondrial genes can be used as a biomarker for neuromuscular dysfunction.

The *BioMedia* Group continues to rely on Chris Weidler (Farmingdale State College) and his range of computer skills. Chris created all of the themes for our new blogs and has stepped in to help with video processing. Tony Biondo (Stony Brook University) provided vital programming support on our *iPlant DNA Subway*, as well as video encoding for hundreds of online clips.

We bid farewell to a number of interns when they began their college careers: Charmaine Brown (St. John's University), Nancy Desai (Boston University), Rachel Gellerman (Binghamton University), Yasmina Macer (Wellesley College), Stephanie Parascandalo (Queens College), Arielle Scardino (City College of New York), and Kaitlin Watrud (Gettysburg College). A number returned to assist with summer camps: Nick Wilkens (Ithaca College), Seth Schortz (Emory University), Lauren Thompson (Barnard College), Matthew Woo (Vassar College), and Janice Yong (Boston University).

Expert Advisors and Corporate Support

We are lucky to have high-level support from two advisory bodies: the Dolan DNA Learning Center Committee and the Corporate Advisory Board (CAB). The DNALC Committee consists of community leaders and members of the CSHL senior management and Board of Trustees, who oversee strategic development, including capital funding and the evolution of satellite locations in North America and beyond. The CAB provides liaison to the Long Island and New York City business communities; its annual fund campaign and golf tournament contributed $225,000 in 2009.

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David A. Micklos, Executive Director, Dolan DNA Learning Center
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Kurt Timmel, Marsh USA
Jeffrey K. Tupper, U.S. Trust Company of New York
Robert Van Nostrand
Hans Zobel, Ziehm Medical LLC

2009 Workshops, Meetings, and Collaborations

January 8	*iPlant Collaborative* Cyberinfrastructure Workshop, Biosphere 2, University of Arizona, Tucson, Arizona
January 8	National Genographic Presentation, Nassau BOCES, Garden City, New York
January 10	HHMI Professional Development Workshop, "PCR and Human DNA Variations, Part 2," *Harlem DNA Lab*
January 12	Site visit by *G2C Online* Fellow Caren Gough, Education Consultant
January 23	NIH *Inside Cancer* Workshop, Illinois Institute of Technology, Chicago, Illinois
January 24	*Saturday DNA!* "Murder at the Museum," DNALC
January 27	Meeting with Scott Bronson, Brookhaven National Laboratory, Upton, New York
February 3	Site visit by Robin Hood Foundation, *Harlem DNA Lab*
February 6	Site visit by Marc Nivet, Josiah Macy, Jr. Foundation, New York
February 9	Site visit by Wanda Wakal, Executive Officer, Research & Development in Gifted & Enrichment, Learning Support Organization, *Harlem DNA Lab*
February 9	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
February 14	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
February 17–18	*G2C Online* Workshop, Contra Costa County School District, Pleasant Hill, California
February 19	NIH *Inside Cancer* Workshop, Contra Costa County School District, Pleasant Hill, California
February 19	Site visit by *G2C Online* Fellows Caren Gough, Education Consultant, and Laura Maitland, Advanced Placement Psychology Consultant
February 21	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
February 24	Site visit by Zoraya Victory, World Science Festival Planning Committee, *Harlem DNA Lab*
February 26	Science Supervisors' Meeting with Alan Ascher, *Harlem DNA Lab*
February 27	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
February 28	*Saturday DNA!* "Dust Away Crime," DNALC
March 5	Site visit by Dale Cole with OSI Pharmaceuticals Inc. members and students from Wyandanch schools
March 6–7	HHMI Peer Cluster Meeting, University of Wisconsin, Milwaukee, Wisconsin
March 6–7	*G2C Online* Workshop, *Harlem DNA Lab*
March 9–10	*G2C Online* Workshop, Fralin Biotechnology Center, Blacksburg, Virginia
March 10	Site visit by Abby Kirschner, Curriculum and Content Coordinator, NYC Teaching Fellows Program, *Harlem DNA Lab*
March 12	HHMI Professional Development Workshop, "Transformation and Protein Isolation," *Harlem DNA Lab*
March 12	NIH *Inside Cancer* Workshop, Virginia Tech, Blacksburg, Virginia
March 13	Site visit by Orla Dolan, Cork Cancer Research Center, Cork, Ireland
March 13	Site visit by Ryan Goble, Curriculum Coordinator, Literacy and New Teacher Coach, Banana Kelly High School, *Harlem DNA Lab*
March 13–14	NSF *Silencing Genomes* Follow-up Workshop, Virginia Tech, Blacksburg, Virginia
March 19–21	National Science Teachers Association National Conference Presentations: "Bioinformatics in Your Classroom," "Sense in Molecules: A Polymorphic Analysis," and "*G2C Online*," New Orleans, Louisiana
March 20	Site visit by David Porteous, University of Edinburgh Centre for Molecular Medicine and Institute of Genetics and Molecular Medicine, Edinburgh, Scotland
March 20	Women in Science Conference, Suffolk Community College, Brentwood, New York
March 21	HHMI Professional Development Workshop, "Transformation and Protein Isolation," *Harlem DNA Lab*
March 28	*Saturday DNA!* "Mitochondrial DNA," DNALC
March 28	Site visit by College of New Rochelle alumni, New Rochelle, New York
March 28	NIH *Inside Cancer* Workshop, *Harlem DNA Lab*
April 4	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
April 17	Interview with Sue Wessler, University of Georgia, Athens, Georgia, DNALC
April 17	NIH *Inside Cancer* Workshop, University of Colorado Anschutz Medical Campus, Denver, Colorado
April 18	*G2C Online* Workshop, University of Colorado Anschutz Medical Campus, Denver, Colorado
April 20–23	Site visit to Carolina Biological Supply Company, Burlington, North Carolina
April 22–24	Seminar, workshop, and dedication of *MobileLab*, Ho Yu College and Primary School, Hong Kong
April 25	NIH *Inside Cancer* Workshop, Arizona State University, Tempe, Arizona
April 25	Science Council of New York City Workshop, Stuyvesant High School, New York
May 1	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
May 1–2	*G2C Online* Workshop, Arizona Biodesign Institute, Arizona State University, Tempe, Arizona
May 5	Site visit by Jan Kang, Trinity School, *Harlem DNA Lab*
May 7	*Great Moments in DNA Science* Honors Seminar: "Of Mice and Men: Using a Mouse Model to Identify New Targets for Cancer Therapy," Eric Sawey, CSHL
May 8	Interview with Svante Pääbo, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany, DNALC

May 9	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part One," *Harlem DNA Lab*
May 14	*Great Moments in DNA Science* Honors Seminar: "Using High-throughput Sequencing to Study Cancer Genomes," Emily Hodges, CSHL
May 15	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part One," *Harlem DNA Lab*
May 16	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part Two," *Harlem DNA Lab*
May 16	*Saturday DNA!* "Who's the Suspect?" DNALC
May 18–20	NIH SEPA Principal Investigators Meeting, Science Museum of Minnesota, St. Paul, Minnesota
May 21	*Great Moments in DNA Science* Honors Seminar: "Illuminating Biology—The Science of Fluorescent-tagged Proteins in Corn," Stacy DiBlasio, CSHL
May 21–22	NSF *Silencing Genomes* Follow-up Workshop, Austin Community College, Austin, Texas
May 22	NIH *Inside Cancer* Workshop, Minneapolis Community & Technical College, Minneapolis, Minnesota
May 22	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part Two," *Harlem DNA Lab*
May 26	North Carolina Life Science and Bioinformatics Education Symposium, North Carolina Biotech Center, Raleigh, North Carolina
May 28	Interviews with Russell Doolittle, Harvard University, Cambridge, Massachusetts; Craig Venter, J. Craig Venter Institute, San Diego, California; and Tim White, University of California, Berkeley, DNALC
May 29	Interviews with Seth Grant, Wellcome Trust Sanger Institute, Cambridge, England; Nicole King, University of California, Berkeley; and Sue Wessler, University of Georgia, Athens, DNALC
May 30–31	NSF *Silencing Genomes* and *G2C Online* Seminars, Bossier Parish Community College, Bossier Parish, Louisiana
May 31	NIH *Inside Cancer* Workshop, Bossier Parish Community College, Bossier Parish, Louisiana
June 1	Interviews with John Doebley, University of Wisconsin, Madison, and Eugenie Scott, National Center for Science Education, Oakland, California, DNALC
June 1–5	NSF *Silencing Genomes* Workshop, California State University, Dominguez Hills, California
June 6	NIH *Inside Cancer* Workshop, St. Louis Science Center, St. Louis, Missouri
June 8–12	NSF *Silencing Genomes* Workshop, St. Louis Science Center, St. Louis, Missouri
June 9	16th Annual Golf Outing, Piping Rock Club, Locust Valley, New York
June 9	Site visit by Kristin Baldwin and Amy Chase, *E. coli* Art Project for World Science Festival, *Harlem DNA Lab*
June 10	NIH *Inside Cancer* Workshop, Tulsa Community College, Tulsa, Oklahoma
June 10	Site visit by Kidgie Williams, Hospitality Committee for United Nations Delegations, Inc., and United Nations delegates' family members, New York
June 11	Site visit by Maxmillian Angerholzer III, Executive Director and Secretary, Lounsbery Foundation, *Harlem DNA Lab*
June 11	World Science Festival, New York University, New York
June 11–12	*G2C Online* Workshop, Tulsa Community College, Tulsa, Oklahoma
June 12	Site visit by Kristin Baldwin and Amy Chase, *E. coli* Art Project for World Science Festival, *Harlem DNA Lab*
June 13	*Saturday DNA!* "Oil Eating Bacteria," DNALC
June 15–19	*DNA Science* Workshop, *Harlem DNA Lab*
June 17–19	*Genomics in Education* Symposium, St. Louis, Missouri
June 20	NIH *Inside Cancer* Workshop, North Carolina Agricultural & Technical University, Greensboro
June 22–26	*Fun with DNA* Workshop, *Harlem DNA Lab*
June 22–26	NSF *Silencing Genomes* Workshop, North Carolina Agricultural & Technical University, Greensboro
June 29	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
June 29–July 2	*Fun with DNA* Workshop, DNALC *Fun with DNA* Workshop, DNALC *West* *Plant Genomics* Workshop, DNALC *World of Enzymes* Workshop, DNALC
June 30	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
July 1	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part One," *Harlem DNA Lab*
July 2	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part Two," *Harlem DNA Lab*
July 6	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
July 6–10	*DNA Science* Workshop, DNALC *Fun with DNA* Workshop, DNALC *Green Genes* Workshop, DNALC *World of Enzymes* Workshop, DNALC *West*
July 7	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
July 8	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part One," *Harlem DNA Lab*
July 9	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part Two," *Harlem DNA Lab*
July 9	Site visit by New York State Biology Professional Development Network with Caren Gough, Education Consultant
July 10	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
July 13	Site visit by Tuan Chiong Chew, Executive Director, Singapore Science Center, Singapore

July 13–17	*DNA Science* Workshop, DNALC *West*
	Genetic Horizons Workshop, DNALC
	World of Enzymes Workshop, DNALC
July 13–24	Site visit by *Inside Cancer* Faculty Fellows Wendy Wooten, Robin Cochran-Dirksen, and Greg Ballog
July 13–24	HHMI New York Leadership Symposium, DNALC
July 15	Site visit by Rod Miller, Mark Greenburg, Donna Murasko, and Patricia Austin, Drexel University, Philadelphia, Pennsylvania
July 15–17	American Association for the Advancement of Science Meeting, "Transforming Undergraduate Biology Education," Washington, D.C.
July 16	Site visit by NYS Assembly member Deborah J. Glick, Greenwich Village, New York
July 17	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
July 20–24	*Fun with DNA* Workshop, DNALC
	Green Genes Workshop, DNALC *West*
	Human Genomics Workshop, DNALC
July 20–31	Site visit by *Inside Cancer* Fellows Lisa Orenstein and Maggy Witecki
July 21	Site visit by Lawrence Kobilinsky, Albert Harpe, and Linda Rourke, John Jay College of Criminal Justice, New York
July 22	Site visit by Rick Acritelli and 10 teachers, The New York Center for Teacher Development, Port Jefferson, New York
July 24	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
July 24	Site visit by 15 STEP program students, SUNY, Old Westbury, New York
July 27–31	*DNA Science* Workshop, *Harlem DNA Lab*
	Genetic Horizons Workshop, DNALC *West*
	Green Genes Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Human Genomics Workshop, DNALC
July 31	NIH *Inside Cancer* Workshop, Howard University, Washington, D.C.
August 3	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
August 3–7	NSF *Silencing Genomes* Workshop, Howard University, Washington, D.C.
August 3–7	*DNA Science* Workshop, DNALC
	Fun with DNA Workshop, DNALC
	Fun with DNA Workshop, DNALC *West*
	Genetic Horizons Workshop, DNALC
August 4	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
August 5	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
August 6	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
August 7	*G2C Online* Workshop, Madison Area Technical College, Madison, Wisconsin
August 8	NIH *Inside Cancer* Workshop, Madison Area Technical College, Madison, Wisconsin
August 10–14	*DNA Science* Workshop, DNALC
	Green Genes Workshop, DNALC
	Human Genomics Workshop, *Harlem DNA Lab*
	World of Enzymes Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
August 12–14	Professional Development Workshop, Hauppauge UFSD, New York
August 14	NIH *Inside Cancer* Workshop, Houston Community College Northwest, Houston, Texas
August 17	Site visit by Jill Hirsch, Kathy Tompkins, and Lacey Tompkins, The Chapin School, New York
August 17–20	HHMI Peer Cluster Meeting, Great Falls, Montana
August 17–21	*DNA Science* Workshop, DNALC
	DNA Science Workshop, DNALC West
	Fun with DNA Workshop, DNALC
	Fun with DNA Workshop, *Harlem DNA Lab*
	Genetic Horizons Workshop, DNALC
August 17–21	NSF *Silencing Genomes* Workshop, Houston Community College Northwest, Houston, Texas
August 24	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
August 24–28	*DNA Science* Workshop, DNALC
	DNA Science Workshop, Wyandanch Public Library, Wyandanch, New York
	Green Genes Workshop, DNALC
	Human Genomics Workshop, DNALC *West*
	World of Enzymes Workshop, DNALC
August 25	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
August 26	Site visit by HHMI Fellow Caren Gough, Executive Consultant, and Kathleen Rucker, Brooklyn International High School, Brooklyn, New York

August 26	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
August 27	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
August 28	*G2C Online* Workshop, John Jay College of Criminal Justice, New York
August 29	NIH *Inside Cancer* Workshop, John Jay College of Criminal Justice, New York
Aug. 31–Sept. 4	*DNA Science* Workshop, DNALC *Fun with DNA* Workshop, DNALC *Green Genes* Workshop, DNALC *West* *World of Enzymes* Workshop, DNALC
September 1	Site visit by Hilleary Osheroff, Samara Rubinstein, and Monique Scott, American Museum of Natural History, New York
September 8	Site visit by Scott Bronson, Safiyh Taghavi, and Daniel van der Lelie, Brookhaven National Laboratory, Upton, New York
September 9	Site visit by Joan Kiely, Stony Brook University, Stony Brook, New York
September 11–18	JGI Metagenomics Course, Walnut Creek, California
September 16	Site visit by 10 scientists, Tsinghua University, Beijing, China
September 21	Interview with Jean-Philippe Vielle Calzada, Laboratorio Nacional de Genomica para LaBiodiversidad, Irapuato, Mexico, DNALC
September 21	Meeting with Bhuma Krishnamachari, C.W. Post Genetic Counseling Program, C.W. Post University, Brookville, New York
September 24	Interview with Janet Rossant, The Hospital for Sick Children, Toronto, Canada, DNALC
September 24	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
September 25	Site visit by Hilleary Osheroff, American Museum of Natural History, New York, New York, *Harlem DNA Lab*
September 26	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
October 3–9	*Weed to Wonder* Videography, Balsas River Valley region of Mexico, documenting the history of maize cultivation
October 5	Interview with Suketoshi Taba, International Maize and Wheat Improvement Center, Texcoco, Mexico
October 7	Interviews with Jean-Philippe Vielle Calzada and Luis R. Herrera Estrella, Laboratorio Nacional de Genomica para La Biodiversidad, Irapuato, Mexico
October 9	NIH *Inside Cancer* Workshop, Oxnard College, Oxnard, California
October 10	*G2C Online* Workshop, Oxnard College, Oxnard, California
October 16	Interview with Ed Buckler, Cornell University, Ithaca, New York, and Torbert Rocheford, Purdue University, West Lafayette, Indiana, DNALC
October 17	*Saturday DNA!* "Disease Detectives," DNALC
October 17	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
November 2	*Weed to Wonder* videography of George Shull Recreation, Uplands Farm, CSHL
November 2–4	Site visit by Maria Halaschek-Weiner and Jochen Stadler, Vienna Open Lab, Vienna, Austria
November 3	HHMI Professional Development, "Bacterial Transformation and Protein Isolation," *Harlem DNA Lab*
November 5	NIH *Inside Cance*r Workshop, Raritan Community College, Somerville, New Jersey
November 6	*G2C Online* Workshop, Raritan Community College, Somerville, New Jersey
November 7	HHMI Professional Development Workshop, "Bacterial Transformation and Protein Isolation," *Harlem DNA Lab*
November 9–10	HHMI Precollege Programs Meeting, Bethesda, Maryland
November 10–13	NSF Research Coordination Networks–Undergraduate Biology Education Grant Review, Arlington, Virginia
November 14	*Saturday DNA!* "Personalized Medicine," DNALC
November 14	HHMI Professional Development Workshop, "Bacterial Transformation and Protein Isolation," *Harlem DNA Lab*
November 20	Site visit by Richard Thompson, Melanie Harasym, and alumni, College of New Rochelle, New Rochelle, New York, with Barbara Candee, CSHL Association Board Director
November 20	*G2C Online* Workshop, Great Bay Community College, Portsmouth, New Hampshire
November 21	NIH *Inside Cancer* Workshop, Great Bay Community College, Portsmouth, New Hampshire
November 30	Meeting with middle and high school science faculty, Trinity School, *Harlem DNA Lab*
Nov. 30–Dec. 11	Singapore Primary Teachers Attachment, DNALC
December 2–4	NSF ATE Grant Review Panel, National Science Foundation, Arlington, Virginia
December 3	Site visit by Barbara Gill, Barbara Rich, and Janet Eilber, The Dana Foundation, New York
December 4	Meeting with Robert Krulwich, National Public Radio, New York
December 5	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
December 12	*Saturday DNA!* "Life Up Close: Microscopic Worlds," DNALC
December 14	Site visit by Theresa Regnante, United Way of Long Island, and Teresa Kemp-Zielenski, DNALC Corporate Advisory Board member
December 19	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part One," *Harlem DNA Lab*
December 30	Site visit by Andrea and Sabrina Gallego, The Simons Foundation, New York

Sites of Major Faculty Workshops 1985–2009

Key: *Middle School* High School **College**

ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	University of Alaska, Fairbanks	1996
ARIZONA	Arizona State University, Tempe	2009
	Tuba City High School	1988
ARKANSAS	Henderson State University, Arkadelphia	1992
CALIFORNIA	**California State University, Dominguez Hills**	**2009**
	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, 2009
	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
	Oxnard Community College, Oxnard	2009
	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, 2009
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
ILLINOIS	Argonne National Laboratory	1986–1987
	Illinois Institute of Technology, Chicago	2009
	University of Chicago	**1992, 1997**
INDIANA	Butler University, Indianapolis	1987
IDAHO	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Bossier Parish Community College	2009
	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	Minneapolis Community and Technical College	2009
	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**	**2008–2009**
	Stowers Institute for Medical Research, Kansas City	2002, 2008
	Washington University, St. Louis	**1989, 1997**
NEVADA	University of Nevada, Reno	1992
NEW HAMPSHIRE	Great Bay Community College, Portsmouth	2009
	New Hampshire Community Technical College, Portsmouth	**1999**
	St. Paul's School, Concord	1986–1987
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
	Raritan Valley Community College, Somerville	2009
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, 2006–2009
	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	2008–2009
	Huntington High School	1986
	Irvington High School	1986
	John Jay College of Criminal Justice	2009
	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, Lake Saranac	2001
	Union College, Schenectady	2004
	United States Military Academy, West Point	**1996**
	Wheatley School, Old Westbury	1985

NORTH CAROLINA	CIIT Center for Health Research, Triangle Park	2003
	North Carolina Agricultural & Technical State University, Greensboro	**2006–2007, 2009**
	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	Tulsa Community College, Tulsa	2009
	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-Rio Grande Campus	2000
	Austin Community College–Eastview Campus	**2007–2009**
	Houston Community College Northwest, Houston	**2009**
	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, AG 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, 2008–2009**
WASHINGTON	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001, 2008
	University of Washington, Seattle	**1993, 1998**
WASHINGTON, D.C	**Howard University**	**1992, 1996, 2009**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Blood Center of Southeastern Wisconsin, Milwaukee	2003
	Madison Area Technical College	**1999, 2009**
	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
CHINA	**Ho Yu College, Hong Kong**	**2009**
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, Merida	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
THE NETHERLANDS	**International Chromosome Conference, Amsterdam**	**2007**

COLD SPRING HARBOR
LABORATORY PRESS

2009 PUBLICATIONS

SERIALS

Genes & Development, Vol. 23 (www.genesdev.org)
Genome Research, Vol. 19 (www.genome.org)
Learning & Memory, Vol. 16 (www.learnmem.org)
RNA, Vol. 15 (www.rnajournal.org)
Cold Spring Harbor Symposia on Quantitative Biology, Vol. 73: *Control and Regulation of Stem Cells,* edited by Bruce Stillman, David Stewart, and Terri Grodzicker
Cold Spring Harbor Protocols (www.cshprotocols.org)
Cold Spring Harbor Perspectives in Biology (www.cshperspectives.org)

LABORATORY MANUALS

Genetics of Complex Human Diseases: A Laboratory Manual, edited by Ammar Al-Chalabi and Laura Almasy
Live Cell Imaging, 2nd ed.: A Laboratory Manual, edited by Robert D. Goldman, Jason R. Swedlow, and David L. Spector
Mouse Hematology: A Laboratory Manual, by Michael P. McGarry, Cheryl A. Protheroe, and James J. Lee

HANDBOOKS

Statistics at the Bench: A Step-by-Step Handbook for Biologists, by Martina Bremer and Rebecca W. Doerge

TEXTBOOKS

Untangling the Double Helix: DNA Entanglement and the Action of the DNA Topoisomerases, by James C. Wang

MONOGRAPH

The Skeletal System, edited by Olivier Pourquié

SCIENCE AND SOCIETY

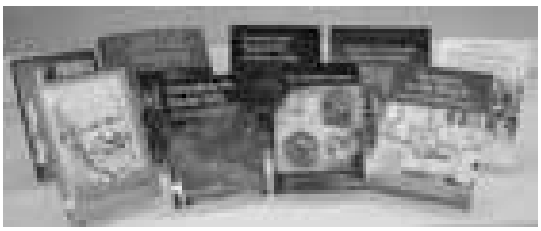
What a Time I Am Having: Selected Letters of Max Perutz, by Vivien Perutz
Francis Crick: Hunter of Life's Secrets, by Robert Olby

OTHER

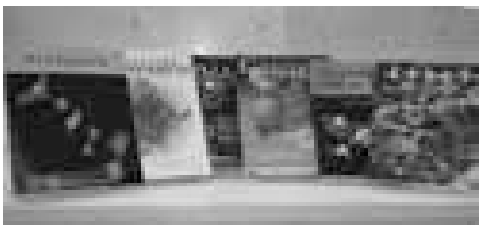
CSHL Annual Report 2008; Yearbook Edition
CSHL Annual Report 2008
Banbury Center Annual Report 2008
Watson School of Biological Sciences Annual Report 2008

WEBSITES

http://cshmonographs.org/index.php/monographs; Cold Spring Harbor Monographs Archive Online
http://www.cshl.symposium.org; Symposia, Vol. 73 added to online Symposia website



A selection of recently published books



The journal publishing program

CSHL Press' new online journal, *Perspectives in Biology*

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

Cold Spring Harbor Laboratory's outstanding research activities are complemented by a renowned program of professional and public education in biomedicine that is transmitted worldwide by Cold Spring Harbor Laboratory Press. Its evolution into a well-known and respected professional publisher has produced six research journals, two of them among the most highly ranked in science; the world's most widely used laboratory manuals; prestigious monographs; an outstanding series of conference proceedings; and best-selling primers on professional skill development for scientists. These activities enhance the work of other educational divisions, produce revenue that supports the Laboratory's work as a whole, and strengthen Cold Spring Harbor's association with excellence.

In 2009, for the sixth consecutive year, the Press generated a substantial operating excess, with a margin of $516,000 that exceeded budget by $100,000, on revenues of $9.1 million. The Press' total contribution to the Laboratory, including support for institutional overhead, was $1.1 million.

Journals

Revenue from journal publishing remains vital to the financial health of the Press. In 2009, economic recession overtook most of the countries that provide the lion's share of that revenue. Nevertheless, gratifyingly, the year proved to be a solid one for journal subscriptions, with four of our five titles meeting or exceeding their sales targets. The exception was our newest journal *Cold Spring Harbor Protocols*, which fell short of a notably aggressive projection but nonetheless advanced strongly. Journal purchasing decisions are made in the third quarter of the year preceding the subscription year, so the full impact of recession on library budgets may not be seen until subscriptions are renewed for 2011. To assist struggling institutions, and as a protective measure, price increases for journals in 2010 were restricted to less than 4%. Initiatives to support retention and growth include new subscription packages offering discounts to libraries subscribing to all Press titles. Proactive promotions of subscription renewals, including journal packages with our newest titles, began earlier than usual, with promising results. Proposals for multilibrary subscription packages in the most recent quarter include offers to groups in India, Hong Kong, Australia, Brazil, Korea, Japan, and France. Although such proposals usually take many months or years to conclude, we have recently signed agreements in India and France and anticipate more success in 2010.

Advertising sales in the journals also proved pleasingly robust despite general market gloom and mergers in the laboratory equipment and reagents market that continued to reduce the client base. Print remains the main driver of advertising sales, but there is a gradual and persistent climb in revenue from electronic placement.

Subscription growth and advertising sales performance are highly dependent on the journals' editorial value to the community. The metrics of value are under scrutiny and the long-established "impact factor" is no longer either the sole, or unassailable, arbiter of quality. But by all available measures, our journals *Genes & Development* and *Genome Research* continue to rank at the peak of distinction in the fields of genetics and developmental and cell biology. *Learning & Memory*, a specialized journal in the vast field of neuroscience, continues to attract a substantial number of submissions and appears to have made an untroubled transition to an "online-only" condition with only a minimal loss of subscriptions. *RNA*, the journal published for The RNA Society, also continued to be editorially vigorous, assisted by the revitalization of RNA biology, and as the year ended, we were delighted by the Society's declaration of its wish to continue a publishing relationship with the Press beyond the end of the current contract in December 2010. The excellence of our production, marketing, and editorial

services and sound strategic guidance were cited by the Society as the reasons for their decision, and a plan is being devised to bring further improvements to the journal.

Toward the year's end, *Cold Spring Harbor Protocols*, our methods journal, achieved acceptance for indexing in Medline, the premier reference source in the life sciences, recognition that will enable this still-growing journal to gain additional traffic and exposure.

In December, the long-standing European editorial office of *Genes & Development* began winding down, in order to consolidate all manuscript review and selection in the Cold Spring Harbor office. This decision, taken with intent to increase efficiency and cost effectiveness in full consultation with co-owner The Genetics Society of Great Britain, alters an editorial structure put in place at the journal's launch 22 years ago, when the scientific community was more circumscribed and the technologies underlying journal publishing were dramatically different. Now that papers have global origins and the entire editorial process can be conducted electronically, from manuscript submission through the review of proofs, there is no longer the same need for a regional office. Since the journal began, five scientists have been European Editor: Graham Bulfield, Nicholas Hastie, Davor Solter, Rudi Grosschedl, and Winship Herr. They have been important to the journal's emergence as one of the most significant in biological science and we are most grateful for their service. With the increase in activity required at Cold Spring Harbor in 2010, the editorial team will be expanded.

The globalization of bioscience is underscored by the Laboratory's ambitious new conference center at Dushu Lake, Suzhou, China. Methods of promoting our publications there are being explored and local printing of sample issues, regional advertising partnerships, and subject-specific content collections are some of the possibilities under consideration.

The launch in July of our newest serial, the review journal *Cold Spring Harbor Perspectives in Biology*, after an 18-month development period, showcased the exceptional quality to be expected from this journal. Its unique feature is its publication of collections of articles that have each been commissioned to provide an in-depth, coherent survey of a single topic. More than 20 subject collections from cell and molecular biology are in the publication pipeline, and each collection has commissioning editors with outstanding international reputations. Each article is being published individually in an issue of the journal, but when complete, the collections will be available in print or as downloadable electronic books. The journal is available free of charge for a year before fees are imposed for access.

Mark Beavers (*left*), conference coordinator for CSHL's Meeting and Courses department, and David Crotty (*right*), executive editor of *CSH Protocols*, at the Cold Spring Harbor booth at the October 2009 Society for Neuroscience meeting in Chicago.

Press Marketing Manager Ingrid Benirschke (*left*) overseeing the Cold Spring Harbor booth at the October 2009 Society for Neuroscience meeting in Chicago.

The response from scientists to the *Perspectives* concept has been so positive that recruiting has begun for *Cold Spring Harbor Perspectives in Medicine*, a source of research information for physicians and scientists working in cancer, neuropathies, psychiatric disorders, infectious disease, metabolic syndrome, and other disorders. This publication will appear first as a supplement, becoming a separate publication and additional source of revenue later.

Other Online Publications

A CSHL Press Monograph online archive was launched in October and features the series' entire collection of titles from 1972 to 2009. The archive's 59 full-text volumes will be sold to institutions as a complete collection and to individuals on a pay-per-chapter basis. Online development and publication were accomplished with modest investment, in collaboration with The Public Knowledge Project at Vancouver's Simon Fraser University. This is the second archive of classic publications from the Laboratory to be made available, following last year's release of the complete electronic archive from 1933 of the Annual Symposia.

In trade publishing, 2009 was the year that the e-book became a commercial reality. The mass media were abuzz with the explosion in sales of handheld reading devices such as the Kindle and Sony Reader. iPhone users made book readers their favorite applications, and sales of e-books rose sharply at Amazon.com. Amid the frenzy, it was necessary to remember that only about 2% of all book sales were e-books, that fewer than 3 million Kindles had been sold, and that Amazon was controlling what purchasers paid for an e-book, regardless of the publisher's price. Sales of Cold Spring Harbor's six Kindle editions were low, as expected, because the device is too expensive for most scientists, and the most popular title, unsurprisingly, was our single work of fiction, *Experimental Heart*. But the experience of producing and marketing books in this form was valuable, because the demand and technologies for electronic books are certain to evolve rapidly in 2010. The Press is responding with several new initiatives. These include the sale of e-books directly from the Press website, the creation of content applications for mobile devices including the iPad and iPhone, Google Editions as a possible sales partner, changes in the business model of sales to the Kindle platform, the creation of device-readable editions of our journals, and the design of dedicated, browsable multimedia sites for certain key book titles. Our goal is to cost effectively make our content available in as many ways as readers require.

Books

Nine new books were published in 2009 and 17 new contracts were signed with editors and authors. As the list below indicates, the newly published titles included technical manuals, a monograph, an advanced textbook, two volumes of biography, and a handbook on statistical methods that already promises to be one of the strongest book titles the Press has published in recent years. The appearance of the long-awaited scientific biography of Francis Crick by the distinguished historian Robert Olby was a notable event and reviews of the book have been warm and appreciative.

The year's best-selling book titles were *Molecular Cloning, At the Bench, Essentials of Glycobiology, Evolution*, and *Epigenetics*. *Evolution*, the textbook published in July 2007, continues to capture adoptions for classes at universities around the world. The book has been critically acclaimed as the first to successfully integrate evolutionary biology with molecular biology and genomics. Work is now beginning on a second edition and a shorter, nonmajors edition, and the book is being translated into Chinese, Japanese, and Greek languages.

Other translation contracts included a Russian edition of *Epigenetics*, Arabic translation of *The Eighth Day of Creation*, German translation of *Proteomics*, Japanese translation of *Essentials of Glycobiology*, and Indonesian translation for our children's books series *Enjoy Your Cells*.

The decade-long digital transition in the distribution and purchase of scientific information continues to drive the evolution of our marketing and sales strategies. A monthly electronic newsletter is sent to more than 45,000 individuals worldwide each month, promoting new titles, journal articles, and the work of the other educational divisions of the Laboratory. A new quarterly e-newsletter to librarians has also proved useful. The addition of features and functions to our website continues to enhance its value as the cornerstone of our presence in the information landscape.

Our publishing program benefits greatly from the local expertise of commission-based sales agents in China, Japan, India, and South America. In recent years, European book sales and marketing have been managed in the same way, but from 2010, these functions will return to the direct control of the Press. Warehousing, order processing, and book distribution will be handled by a U.K. company, and a part-time marketing manager has been appointed to complement and enhance the activities of the Cold Spring Harbor sales and marketing team. This reorganization saves significant expense, allows expansion of our marketing activities, and connects us more directly with customers in our largest non-U.S. market.

Staff

Two promotions were announced during the year: Laureen Connell from Assistant to Associate Editor of *Genes & Development*, and Christina Lo to the additional position of Contracts and Rights Manager. We also said farewell to Lauren Schmidt and welcomed Ann Smith as her replacement. Dr. Nicholas Oswald was appointed European Marketing and Sales Manager, based in Edinburgh U.K.

A complete list of staff members of the Press as of December 2009 is printed elsewhere in this volume. The Laboratory is fortunate to have the service of such capable and dedicated professionals. Special thanks are due to those with the added responsibility of leadership in the diverse departments and projects of the Press: Jan Argentine, David Crotty, Alex Gann, Terri Grodzicker, Christina Lo, Geraldine Jaitin, Bill Keen, Wayne Manos, Richard Sever, Marcie Siconolfi, Hillary Sussman, Linda Sussman, and Denise Weiss.

John R. Inglis
Executive Director
and Publisher

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2009
(with comparative financial information as of December 31, 2008)

	2009	2008
Assets:		
Cash and cash equivalents	$ 65,950,558	52,226,592
Grants receivable	6,200,398	8,597,399
Contributions receivable, net	119,740,185	104,681,657
Publications inventory	4,570,278	3,925,967
Investments	241,595,564	213,424,159
Restricted use assets	2,111,090	20,947,929
Deposits with bond trustee	–	11,886,744
Other assets	10,398,213	10,227,962
Land, buildings and equipment, net	237,791,406	198,684,203
Total assets	$ 688,357,692	624,602,612
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 26,475,011	50,563,503
Deferred revenue	5,640,029	3,962,000
Bonds payable	97,200,000	97,200,000
Total liabilities	129,315,040	151,725,503
Net assets:		
Unrestricted	235,125,827	126,126,561
Temporarily restricted	142,357,714	179,988,153
Permanently restricted	181,559,111	166,762,395
Total net assets	559,042,652	472,877,109
Total liabilities and net assets	$ 688,357,692	624,602,612

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2009
(with summarized financial information for the year ended December 31, 2008)

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2009 Total*	*2008 Total*
Revenue and other support:					
Public support–contributions and non-Federal grant awards	$ 10,276,866	54,571,890	2,255,040	67,103,796	102,678,072
Federal grant awards	29,648,968	–	–	29,648,968	27,728,155
Indirect cost allowances	21,016,585	–	–	21,016,585	22,079,594
Investment return utilized	18,216,748	–	–	18,216,748	16,956,184
Program fees	5,484,810	–	–	5,484,810	4,634,167
Publications sales	9,136,188	–	–	9,136,188	10,998,768
Dining services	4,103,820	–	–	4,103,820	4,074,984
Rooms and apartments	3,255,480	–	–	3,255,480	3,088,435
Miscellaneous	1,962,873	–	–	1,962,873	2,300,014
Net assets released from restrictions	48,880,350	(48,880,350)	–	–	–
Total revenue and other support	151,982,688	5,691,540	2,255,040	159,929,268	194,538,373
Expenses:					
Research	73,046,245	–	–	73,046,245	69,062,315
Educational programs	15,319,175	–	–	15,319,175	15,259,681
Publications	9,075,715	–	–	9,075,715	10,764,090
Banbury Center conferences	1,069,044	–	–	1,069,044	1,309,337
Dolan DNA Learning Center programs	1,467,947	–	–	1,467,947	1,476,222
Watson School of Biological Sciences programs	3,595,810	–	–	3,595,810	3,138,774
General and administrative	14,293,070	–	–	14,293,070	14,545,349
Dining services	4,885,369	–	–	4,885,369	5,345,228
Total expenses	122,752,375	–	–	122,752,375	120,900,996
Excess of revenue and other support over expenses	29,230,313	5,691,540	2,255,040	37,176,893	73,637,377
Other changes in net assets:					
Investment return/(loss) excluding amount utilized	7,475,070	4,447,005	12,541,676	24,463,751	(93,289,813)
Change in fair value of interest-rate swap	24,524,899	–	–	24,524,899	(29,526,602)
Write-off of terminated bond insurance	–	–	–	–	(961,438)
Release of temporarily restricted capital funds	47,768,984	(47,768,984)	–	–	–
Increase (decrease) in net assets	108,999,266	(37,630,439)	14,796,716	86,165,543	(50,140,476)
Net assets at beginning of year	126,126,561	179,988,153	166,762,395	472,877,109	523,017,585
Net assets at end of year	$ 235,125,827	142,357,714	181,559,111	559,042,652	472,877,109

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 2009
(with comparative financial information for the year ended December 31, 2008)

	2009	2008
Cash flows from operating activities:		
Increase (decrease) in net assets	$ 86,165,543	(50,140,476)
Adjustments to reconcile increase (decrease) in net assets to net cash provided by operating activities:		
Change in fair value of interest rate swap	(24,524,897)	29,526,602
Depreciation and amortization	9,282,371	7,056,947
Net (appreciation) depreciation in fair value of investments	(36,992,017)	83,339,568
Contributions restricted for long-term investment	(9,121,400)	(28,230,901)
Changes in assets and liabilities:		
Grants receivable	2,397,001	439,731
Contributions receivable, net of financing activities	(18,458,455)	(30,956,222)
Publications inventory	(644,311)	107,133
Other assets	(611,026)	267,791
Restricted use assets	1,456,592	423,656
Accounts payable and accrued expenses, net of financing activities	32,680	4,152,607
Deferred revenue	1,678,029	(41,091)
Net cash provided by operating activities	10,660,110	15,945,345
Cash flows from investing activities:		
Capital expenditures	(48,389,574)	(46,536,698)
Proceeds from sales and maturities of investments	140,454,706	159,193,212
Purchases of investments	(131,634,094)	(122,321,887)
Net change in investment in employee residences	440,775	1,208,853
Net cash used in investing activities	(39,128,187)	(8,456,520)
Cash flows from financing activities:		
Permanently restricted contributions	2,255,040	12,906,255
Contributions restricted for investment in land, buildings and equipment	6,866,360	15,324,644
Decrease (increase) in contributions receivable	3,399,927	(6,632,433)
Decrease in deposits with bond trustee	11,886,744	17,596,724
Increase (decrease) in accounts payable relating to capital expenditures	403,725	(3,114,384)
Decrease (increase) in deposits with swap counterparty	17,380,247	(17,380,247)
Payment of conversion costs on bonds payable	–	(500,700)
Write-off of terminated bond insurance	–	961,438
Net cash provided by financing activities	42,192,043	19,161,297
Net increase in cash and cash equivalents	13,723,966	26,650,122
Cash and cash equivalents at beginning of year	52,226,592	25,576,470
Cash and cash equivalents at end of year	$ 65,950,558	52,226,592
Supplemental disclosure:		
Interest paid	$ 3,180,748	4,153,373
Noncash investing and financing activities:		
Contributed property	$ 98,085	–

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 New York State, private foundations, corporations, and individuals. The following section summarizes funding that occurred during 2009.

GRANTS January 1–December 31, 2009

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2009 Funding*[1]
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Equipment Support	Drs. Martienssen/McCombie	05/14/09 05/13/10	$ 474,500 *[2]
	Dr. Hannon	08/01/09 12/31/09	99,310 *[2]
	Dr. D. Spector	12/17/09 11/30/10	95,137 *[2]
Program Project and Center Support	Drs. Hannon/Krainer/Lowe/Spector/ Stillman/Tansey	01/01/07 12/31/11	4,501,160
	Dr. Stillman–Cancer Center Core	08/15/05 07/31/10	4,126,879
	Dr. Stillman–Cancer Center Core	09/30/09 03/31/11	199,292 *[2]
	Dr. Zador	09/30/09 08/31/11	719,000 *[2]
Cooperative Research Agreement Support[3]	Drs. Gingeras/Hannon	09/27/07 06/30/11	3,600,584
	Dr. Huang	09/08/06 08/31/11	127,814
	Drs. Lowe/Hannon/Hicks/Powers	09/01/09 08/31/14	821,775 *
Contract Support	Drs. Hannon/Lowe	12/31/09 12/31/11	999,980 *
Research Support	Dr. Dubnau	09/15/09 08/31/14	431,667 *
	Drs. Gingeras/Hannon/McCombie	09/30/09 08/31/11	1,473,081 *[2]
	Drs. Gingeras/Hannon/McCombie	09/30/09 08/31/11	1,401,903 *[2]
	Drs. Gingeras/Hannon/McCombie	09/30/09 08/31/11	1,428,205 *[2]
	Dr. Hannon	09/01/09 08/31/13	344,400 *
	Dr. Huang	09/30/09 08/31/11	498,790 *[2]
	Dr. Huang	04/16/09 03/31/11	252,000 *
	Dr. Joshua-Tor	07/01/07 06/30/12	299,954
	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	638,359
	Dr. Lowe	09/15/09 08/31/14	344,400 *
	Dr. Martienssen	09/29/09 08/31/11	336,000 *[2]
	Dr. Martienssen	08/01/07 07/31/11	412,000
	Dr. Mills	12/26/07 11/30/12	348,600
	Dr. Mitra	02/01/08 12/31/10	189,000
	Dr. Mitra	09/30/09 05/31/14	1,048,290 *
	Dr. Mitra	09/30/09 08/31/11	498,858 *[2]
	Dr. Muthuswamy	02/01/09 12/31/13	375,966 *
	Drs. Powers/Lowe/Krasnitz/Sordella	09/29/09 08/31/11	2,363,922 *[2]

[1]Includes direct and indirect costs
[2]Award issued under the American Recovery and Reinvestment Act of 2009
[3]Cooperative research agreement funding amounts include only CSHL portion of award
*New grants awarded in 2009

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2009 Funding*[1]
	Dr. Powers	12/02/06	11/30/11	439,016
	Dr. Sebat	04/01/07	03/31/10	729,402
	Dr. Sebat	09/30/05	07/31/10	415,639
	Dr. Skowronski	09/26/09	08/31/11	9,869 *[2]
	Dr. Skowronski	05/01/08	04/30/13	420,000
	Dr. D. Spector	04/01/90	03/31/11	652,580
	Dr. Stenlund	02/01/08	01/31/12	420,000
	Dr. Stillman	06/01/08	05/31/12	610,717
	Dr. Tansey	05/01/07	05/31/09	29,423
	Dr. Tonks	07/21/06	05/31/10	289,553
	Dr. Van Aelst	07/01/08	03/31/13	378,000
	Dr. Van Aelst	01/01/09	12/31/13	348,600 *
	Drs. Wigler/McCombie	09/30/09	08/31/11	1,384,503 *[2]
	Dr. Zador	09/24/09	08/31/11	90,236 *[2]
	Dr. Zador	09/18/08	07/31/13	357,000
	Dr. Zhang	05/01/08	02/28/11	650,000
	Drs. Zhang/Krainer/McCombie	08/13/07	07/31/11	470,400
Research Subcontracts				
NIH/Allen Institute for Brain Science Consortium Agreement	Dr. Mitra	09/15/09	08/31/10	201,600 *
NIH/Angion Biomedica Corp. Consortium Agreement	Dr. Enikolopov	09/30/09	08/31/11	271,759 *
NIH/Brookhaven National Laboratory Consortium Agreement	Dr. Stillman	09/01/06	08/31/11	38,854
NIH/Columbia University Consortium Agreement	Dr. Wigler	09/01/06	05/31/10	303,489
NIH/Columbia University Consortium Agreement	Dr. Lowe	08/18/06	07/31/11	407,069
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	09/29/11	427,000
NIH/Ludwig Institute for Cancer Research Consortium Agreement	Dr. Zhang	09/29/08	06/30/13	227,961
NIH/Northwestern University Consortium Agreement	Dr. Osten	09/15/09	08/31/11	42,000 *
NIH/Ontario Institute for Cancer Research Consortium Agreement	Dr. Stein	03/01/09	03/31/10	62,609 *
NIH/Paratek Pharmaceuticals, Inc., Consortium Agreement	Dr. Krainer	01/05/09	12/31/10	166,025 *
NIH/Rutgers University Consortium Agreement	Dr. Mitra	12/08/04	11/30/10	185,940
NIH/University of California, San Francisco, Consortium Agreement	Dr. Egeblad	09/01/09	08/31/14	163,501 *
NIH/University of California, Berkeley, National Laboratory Consortium Agreement	Dr. Gingeras	01/01/09	03/31/11	999,423 *
NIH/University of Southern California Consortium Agreement	Drs. Lowe/Sordella	09/28/09	07/31/14	371,637 *
NIH/Vanderbilt University Consortium Agreement	Dr. Tansey	06/01/09	04/30/10	58,152 *
Fellowship Support	Dr. Borges	08/16/09	08/15/12	50,054 *
	Dr. Lazarus	07/15/09	07/14/11	25,176 *

[1]Includes direct and indirect costs
[2]Award issued under the American Recovery and Reinvestment Act of 2009
*New grants awarded in 2009

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2009 Funding*[1]
Graduate Training Support	Dr. Joshua-Tor, Watson School of Biological Sciences	07/01/07	06/30/12	206,330
	Dr. Joshua-Tor, Watson School of Biological Sciences–Supplement	09/01/09	08/31/11	41,266 *[2]
Course Support	Advanced Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging	07/01/98	08/31/10	86,250
	Advanced Techniques in Molecular Neuroscience	07/01/01	06/30/12	96,512
	C. elegans	08/01/98	08/31/11	80,420
	Cell and Developmental Biology of *Xenopus*	04/01/09	03/31/14	59,900 *
	Computational and Comparative Genomics	06/06/91	07/31/13	48,818
	Eukaryotic Gene Expression	01/01/83	03/31/11	112,154
	Imaging Structure and Function in the Nervous System	07/01/01	06/30/12	46,740
	Integrated Data Analysis for High-throughput Biology	09/01/07	04/30/12	59,464
	Molecular Embryology of the Mouse	01/01/83	03/31/11	113,305
	Neurobiology of *Drosophila*	07/01/01	06/30/12	78,326
	Programming for Biology	09/25/09	08/31/14	60,000 *
	Protein Purification and Characterization	01/01/83	03/31/11	69,977
	Proteomics	07/01/03	06/30/11	78,059
	X-ray Methods in Structural Biology	07/01/07	06/30/12	74,812
	Yeast Genetics and Genomics	07/01/07	05/31/10	50,393
Meeting Support	The Ubiquitin Family	04/11/07	03/31/12	5,000
	The Biology of Genomes	04/01/08	03/31/13	37,080
	Channels, Receptors, and Synapses	04/01/06	03/31/11	23,271
	Cell Death	09/14/07	08/31/12	5,000
	74th Symposium: Evolution: The Molecular Landscape	01/01/09	12/31/09	13,000 *
	Molecular and Cellular Biology of Plasminogen Activation	04/01/09	03/31/10	4,000 *
	Telomeres and Telomerase	03/01/09	02/28/10	10,000 *
	Retroviruses	04/01/09	03/31/10	12,000 *
	Network Biology	04/21/09	02/28/14	7,500 *
	Neurobiology of *Drosophila*	07/01/09	06/30/10	23,000 *
	Genome Informatics	07/01/09	06/30/10	22,000 *
	Mechanisms of Eukaryotic Transcription	08/15/09	07/31/10	5,000 *
	Microbial Pathogenesis and Host Response	08/01/09	07/31/10	21,025 *
	Harnessing Immunity to Prevent and Treat Disease	11/06/09	10/31/10	8,000 *
HEALTH RESOURCES AND SERVICES ADMINISTRATION				
Equipment	Dr. D. Spector	09/01/09	08/31/11	706,860 *
NATIONAL SCIENCE FOUNDATION				
Equipment	Drs. Martienssen/McCombie	09/01/09	08/31/12	999,253 *[2]
Multiple Project Award Support	Dr. Jackson	07/01/05	06/30/10	1,069,628
	Dr. Ware	10/01/07	09/30/11	1,669,733

[1]Includes direct and indirect costs
[2]Award issued under the American Recovery and Reinvestment Act of 2009
*New grants awarded in 2009

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2009 Funding*[1]
Research Support	Dr. Jackson	04/01/07	03/31/10	150,000
	Dr. Lippman	09/15/09	08/31/12	159,324 *
	Dr. Ware	06/15/08	05/31/11	243,954
Research Subcontracts				
NSF/Cornell University Consortium Agreement	Drs. Timmermans/Micklos	09/01/08	08/31/12	591,378
NSF/Marquette University Consortium Agreement	Dr. Jackson	08/01/09	07/31/10	54,800 *
NSF/University of Arizona Consortium Agreement	Drs. Stein/Martienssen/Micklos/Vaughn/Ware	02/01/08	01/31/13	1,204,131
NSF/University of California, Berkeley, Consortium Agreement	Dr. Jackson	08/31/06	07/31/11	221,856
NSF/University of California, Davis, Consortium Agreement	Dr. Martienssen	09/01/06	08/31/10	271,715
NSF/University of California, Davis, Consortium Agreement	Dr. Ware	09/01/08	08/31/11	205,976
NSF/Washington University Consortium Agreement	Drs. McCombie/Martienssen/Ware	11/15/05	10/31/09	436,710
Fellowship Support	C. Malone	06/01/07	05/31/10	40,500
	Dr. Eveland	10/01/08	09/30/10	72,000
Undergraduate Training Support	Dr. Jackson, Watson School of Biological Sciences	03/01/09	02/29/12	66,750 *
Course Support	Molecular Techniques in Plant Science	07/01/07	06/30/10	45,540
	Advanced Bacterial Genetics	07/01/09	06/30/14	88,713 *
	Cell and Developmental Biology of *Xenopus*	05/01/07	04/30/10	20,000
	Computational Cell Biology	04/15/08	03/31/11	94,221
Meeting Support	Engineering Principles for Biological Sciences	02/01/09	01/31/10	15,000 *
	Neurobiology of *Drosophila*	05/01/09	04/30/10	15,000 *
	International Workshop on Rapid Release of Prepublication Data	05/01/09	04/30/10	18,500 *
	Plant Genomes	06/15/09	05/31/10	5,000 *
	Yeast Cell Biology	07/01/09	06/30/10	29,845 *
	Eukaryotic DNA Replication	09/01/09	08/31/10	7,000 *
	Eukaryotic mRNA Processing	09/01/09	08/31/10	7,000 *
	EC-US Plant Biotechnology Workshop on Plant Bioinformatics and Databases	12/01/09	11/30/10	47,922 *
	Mechanisms of Eukaryotic Transcription	09/01/09	08/31/10	7,000 *
UNITED STATES DEPARTMENT OF AGRICULTURE				
Research Support	Dr. McCombie	09/11/09	09/10/14	213,052 *
	Dr. Jackson	09/01/08	08/31/11	128,873
Meeting Support	Bovine Genome	09/01/09	08/31/10	2,000 *
UNITED STATES DEPARTMENT OF THE ARMY				
Research Support	Dr. Hannon	09/01/08	08/31/13	635,946
	Dr. Muthuswamy	06/01/08	05/31/13	762,629
	Dr. Powers	07/01/07	06/30/10	168,000

[1]Includes direct and indirect costs
*New grants awarded in 2009

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2009 Funding*[1]
	Dr. Trotman	08/15/09	08/14/12	127,292 *
	Dr. Wigler	09/01/09	08/31/11	2,441,000 *
Fellowship Support	Dr. He	12/15/08	12/14/11	32,400
MISCELLANEOUS SOURCES OF FUNDING				
Equipment Support				
New York State Department of Health	Dr. D. Spector	01/01/09	12/31/09	481,738 *
Eleanor Schwartz Charitable Foundation	Dr. Osten	03/01/09	02/28/10	200,000 *
Program Project Support				
The Leukemia and Lymphoma Society	Dr. Lowe	10/01/08	09/30/13	1,250,000
Pioneer Hi-Bred International, Inc.	Drs. Jackson/Lippman/Martienssen/ Timmermans/Ware	07/01/07	06/30/12	1,600,000
The Simons Foundation/Autism	Dr. Wigler	07/01/08	06/30/11	6,586,312
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				
Alpern Family Foundation Inc.	Dr. Egeblad	12/15/09	12/14/10	1,000 *
American Cancer Society	Dr. Wigler	01/01/05	12/31/10	10,000
	Dr. Wigler	01/01/06	12/31/10	60,000
	Dr. Mills	07/01/06	06/30/10	240,000
American Heart Association	Dr. Furukawa	07/01/09	06/30/13	77,000 *
Anonymous Gift	Dr. Osten	09/01/09	08/31/11	262,088 *
Autism Speaks, Inc.	Dr. Zador	07/01/07	06/30/10	127,500
Mr. Donald E. Axinn	Drs. Hannon/Lowe	01/01/09	12/31/09	250,000 *
Geoffrey Beene Cancer Center/Sloan-Kettering Institute for Cancer Research	Dr. Hicks	08/01/08	07/31/10	42,654
Breast Cancer Help, Inc./Sons of Italy in America	Dr. Sordella	04/01/09	03/31/10	10,000 *
The Breast Cancer Research Foundation	Dr. Wigler	10/01/09	09/30/10	200,000 *
Clear Channel Worldwide (WALK 97.5 FM) Breast Cancer Research Walk	Dr. Egeblad	04/01/09	03/31/10	5,000 *
Coleman Fung Foundation Inc.	Dr. Zador	02/01/07	01/31/10	50,000
The Dana Foundation	Dr. Li	10/01/09	09/30/12	67,000 *
	Dr. Li	01/01/09	12/31/11	200,000 *
Dart Neuroscience LLC	Dr. Zhong	06/01/09	05/31/10	125,000 *
The Kathryn W. Davis Foundation	Dr. Hannon	01/15/07	01/14/11	1,019,655
The Ellison Medical Foundation	Dr. Enikolopov	09/09/09	09/08/13	252,000 *
Entertainment Industry Foundation	Dr. Hannon	11/01/09	10/31/10	250,000 *
The Eppley Foundation for Research, Inc.	Dr. Zhong	12/22/09	12/21/10	50,000 *
Find a Cure Today (F.A.C.T.)	Dr. Egeblad	12/01/09	11/30/10	25,000 *
Douglas and Christine Fox	Dr. Furukawa	12/15/09	12/14/10	1,000 *
The Joni Gladowsky Breast Cancer Foundation	Dr. Egeblad	12/01/09	11/30/10	35,000 *
GlaxoSmithKline	Dr. Powers	10/01/08	09/30/10	1,099,802
Richard and Mindy Gordon	Dr. Egeblad	01/01/09	12/31/09	5,000 *
The Irving A. Hansen Memorial Foundation	Dr. Tonks	07/01/09	06/30/10	10,000 *
Jo-Ellen and Ira Hazan	Dr. Enikolopov	12/01/09	11/30/10	200,000 *

[1]Includes direct and indirect costs
*New grants awarded in 2009

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2009 Funding*[1]
Hearts for Cancer	Dr. Muthuswamy	12/01/07	11/30/09	1,000
	Dr. Egeblad	04/01/09	03/31/10	1,710 *
The International Human Frontier Science Program Organization	Dr. Lippman	07/01/09	06/30/12	100,000 *
Isis Pharmaceuticals, Inc.	Dr. D. Spector	01/01/09	12/31/09	10,000 *
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	494,535
Mara and Thomas Lehrman Charitable Fund	Dr. Mitra	01/01/09	12/31/09	55,000 *
The Long Island 2-Day Walk to Fight Breast Cancer	Dr. Egeblad	11/01/08	10/31/10	20,000
Manhasset Women's Coalition Against Breast Cancer	Dr. Muthuswamy	01/01/09	12/31/09	50,000 *
Carol Marcincuk Fund	Dr. Lucito	01/01/09	12/31/09	4,690 *
In Honor of Carissa Maringo	Dr. Egeblad	10/01/09	09/30/10	4,300 *
The G. Harold and Leila Y. Mathers Charitable Foundation	Dr. Huang	07/01/07	06/30/10	205,913
The Elizabeth McFarland Breast Cancer Fund	Dr. Wigler	01/01/09	12/31/09	61,801 *
Roni and Charles McGuffog	Dr. Furukawa	03/01/09	02/28/10	1,000 *
The McKnight Endowment Fund for Neuroscience	Dr. Osten	08/01/09	07/31/11	100,000 *
The Don Monti Memorial Research Foundation	Dr. Lowe	03/01/09	02/28/10	500,000 *
Louis Morin Charitable Trust	Dr. Kepecs	12/01/09	11/30/10	70,000 *
MSC Biotarget LLC	Dr. Tonks	11/01/09	10/31/10	60,000 *
Muscular Dystrophy Association, Inc.	Dr. Krainer	07/01/07	06/30/10	241,300
The Neuberger Berman Foundation	Dr. Trotman	11/01/09	10/31/10	20,000 *
New York State Department of Health– New York Stem Cell Science (NYSTEM)	Drs. Enikolopov/Koulakov	01/01/09	12/31/11	359,996 *
	Dr. Timmermans	01/01/09	12/31/10	119,234 *
Philips Research North America	Dr. Zhang	01/01/09	12/31/10	180,000 *
	Drs. Lucito/Hicks	04/01/09	03/31/10	565,671 *
Rahn & Bodmer Co.	Dr. Trotman	12/15/09	12/14/10	400 *
Christina Renna Foundation, Inc.	Dr. Van Aelst	01/01/08	12/31/09	17,300
Diane Emdin Sachs Memorial Fund	Dr. Sordella	06/01/09	05/31/10	23,947 *
In Honor of Jacob T. Schwartz	Dr. Wigler	04/01/09	03/31/10	600 *
Judi Shesh Memorial Foundation	Dr. Hannon	07/01/09	06/30/10	10,000 *
The Simons Foundation	Dr. Mills	01/01/07	12/31/09	544,373
	Dr. Huang	10/01/07	09/30/10	440,805
	Dr. Mitra	09/01/09	08/31/11	150,000 *
	Dr. Osten	07/01/09	06/30/11	149,838 *
Mary F. Smith Family Foundation	Dr. Egeblad	11/01/09	10/31/10	2,000 *
Starr Cancer Consortium	Dr. Egeblad	08/01/09	07/31/11	150,000 *
	Dr. Hannon	07/01/08	06/30/10	452,316
	Dr. Lazebnik	08/01/09	07/31/11	180,000 *
	Dr. Lowe	07/01/08	06/30/10	60,000
	Drs. Lowe/Hannon	11/01/09	10/31/10	480,000 *
	Drs. Lucito/Tonks	08/01/09	07/31/11	473,780 *
	Dr. McCombie	07/01/08	06/30/10	300,000
	Dr. McCombie	08/01/09	07/31/11	180,000 *
	Dr. Powers	08/01/09	07/31/11	120,000 *
	Dr. Trotman	08/01/09	07/31/11	150,000 *
	Dr. Wigler	08/01/09	07/31/11	399,900 *
	Dr. Zhang	08/01/09	07/31/11	240,000 *
Swim Across America	Dr. Sordella	11/01/09	10/31/10	50,000 *

[1]Includes direct and indirect costs
*New grants awarded in 2009

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2009 Funding*[1]
The V Foundation	Dr. Trotman	11/01/08	10/31/10	50,000
	Drs. Powers/Sordella	11/01/08	10/31/11	200,000
Waldbaum Foundation	Dr. Egeblad	09/01/08	08/31/09	8,000
West Islip Breast Cancer Coalition for Long Island Inc.	Dr. Egeblad	11/01/09	10/31/10	10,000 *
West Islip Youth Enrichment Services	Dr. Egeblad	10/01/09	09/30/10	5,660 *
Whitehall Foundation, Inc.	Dr. Kepecs	06/01/08	05/31/11	75,000
Wodecroft Foundation	Dr. Li	09/01/09	08/31/10	25,000 *
Women in Science	Dr. Timmermans	01/01/09	12/31/09	48,296 *
Women's Insurance Network of Long Island	Dr. Egeblad	06/01/09	05/31/10	3,000 *
Fellowship Support				
The Abrams Charitable Trust	Dr. Joshua-Tor	05/01/09	04/30/10	1,500 *
The Rita Allen Foundation	Dr. Trotman	09/01/07	08/31/10	100,000
American Association for Cancer Research	Dr. Miething	07/01/09	06/30/10	30,000 *
American Cancer Society	Dr. Karginov	01/01/07	12/31/09	48,000
Australian Government National Health and Medical Research Council	Dr. Dow	02/01/09	01/31/11	51,875 *
Autism Speaks, Inc.	Dr. Xiong	10/01/08	09/30/10	44,000
Arnold and Mabel Beckman Foundation	Watson School of Biological Sciences	09/01/05	08/31/10	350,000
Terri Brodeur Breast Cancer Foundation	Dr. Bialucha	01/01/09	12/31/10	50,000 *
Canadian Institutes of Health Research	Dr. Aznarez	07/01/09	12/31/11	21,768 *
Carnegie Institution	Dr. Lippman	09/01/09	08/31/10	17,539 *
The Mary K. Chapman Foundation	Dr. Xu	04/01/08	03/31/10	85,000
The Jane Coffin Childs Memorial Fund for Medical Research	Dr. Kuhn	07/01/08	06/30/11	46,500
CSHL Association Fellowship	New Investigator Support	01/01/09	12/31/09	280,000 *
Danish Cancer Society	Dr. Jensen	01/01/07	03/10/10	75,000
Englehorn Scholarship Program	Watson School of Biological Sciences	01/01/09	12/31/09	49,980 *
Genentech Foundation	Dr. Joshua-Tor, Watson School of Biological Sciences	07/01/09	06/30/12	55,017 *
The Allen and Lola Goldring Foundation	Dr. Stillman	09/01/09	08/31/10	75,000 *
Lita Annenberg Hazen Foundation	Watson School of Biological Sciences	05/01/08	04/30/18	10,000
Hope Funds for Cancer Research	Dr. Sawey	07/01/09	06/30/11	42,500 *
Human Frontier Science Program	Dr. Canela	05/01/09	04/30/11	47,400 *
	Dr. Kvitsiani	09/01/08	08/31/11	46,400
	Dr. Skopelitis	04/01/09	03/31/11	46,400 *
International Rett Syndrome Foundation	Dr. A. Paul	10/01/09	09/30/11	50,000 *
	Dr. Lin	01/01/09	12/31/10	50,000 *
The Japan Society for the Promotion of Science	Dr. Hige	07/01/08	06/30/10	48,000
	Dr. Kawakami	04/01/08	03/31/10	52,000
Annette Kade Charitable Trust	Watson School of Biological Sciences	04/01/09	03/31/10	60,000 *
The Esther A. and Joseph Klingenstein Fund Inc.	Dr. Kepecs	07/01/09	06/30/12	50,000 *
The Susan G. Komen Breast Cancer Foundation, Inc.	Dr. Anczukow	06/04/09	06/03/12	60,000 *
Life Sciences Research Foundation	Dr. Xu	11/01/09	10/31/12	56,000 *
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
	Dr. Oviedo	01/15/08	01/14/10	50,000
	Dr. Lu	01/15/09	01/14/11	46,000 *
The Sass Foundation for Medical Research, Inc.	Dr. S. Paul	04/01/08	03/31/10	75,000
The Seligson Foundation	Dr. Zuber	01/01/09	12/31/09	75,000 *
Alfred P. Sloan Foundation	Dr. Kepecs	09/16/09	09/15/11	25,000 *

[1]Includes direct and indirect costs
*New grants awarded in 2009

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2009 Funding*[1]
Lauri Strauss Leukemia Foundation	Dr. Miething	04/01/09	03/31/10	15,000 *
The Swartz Foundation	Dr. Zador	01/01/09	12/31/09	165,000 *
	Drs. Koulakov/Kolterman	01/01/09	12/31/09	55,000 *
	Drs. Zador/Reid	01/01/09	12/31/09	55,000 *
	Dr. Zador	01/01/09	12/31/09	15,000 *
Vanderbilt University Medical Center	A. Leung	09/01/09	08/31/10	37,760 *
Vietnam Education Foundation	Dr. Nguyen	08/01/09	07/31/10	31,200 *
Training Support				
Howard Hughes Medical Institute	Undergraduate Research Program	06/01/09	05/31/10	7,600 *
Steamboat Foundation	Undergraduate Research Program	05/01/09	04/30/10	12,000 *
William Townsend Porter Foundation	Undergraduate Research Program	04/01/09	03/31/10	9,100 *
Course Support				
The Autism Speaks Foundation	Workshop on Autism Spectrum Disorders	05/01/07	04/30/11	15,000
Howard Hughes Medical Institute	Neurobiology Course Support	01/01/07	12/31/11	750,000
IAC-USNC/IBRO	Student Support Course Scholarships	04/01/09	03/31/10	6,395
Illumina, Inc.	Advanced Sequencing Technologies and Applications	03/01/09	02/28/10	30,000 *
Nancy Lurie Marks Family Foundation	Workshop on Autism Spectrum Disorders	05/01/07	04/30/11	15,000
Roche Diagnostics Corporation	Advanced Sequencing Technologies and Applications	03/01/09	02/28/10	30,000 *
The Simons Foundation	Workshop on Autism Spectrum Disorders	05/01/07	04/30/11	15,000
Meeting Support				
Abbott Laboratories	Gene Expression and Signaling in the Immune System	10/01/09	09/30/10	3,000 *
The Company of Biologists Limited	Vertebrate Organogenesis	09/01/09	08/31/10	4,738 *
Dow AgroSciences, LLC	Plant Genomes: Genes, Networks, and Applications	04/01/09	03/31/10	5,000 *
Illumina, Inc.	Personal Genomes	09/14/09	09/13/10	4,250 *
	The Biology of Genomes	04/01/09	03/31/10	3,750 *
Merial Limited	Bovine Genome	04/01/09	03/31/10	4,000 *
Novartis Institutes for BioMedical Research, Inc.	Vertebrate Organogenesis	09/01/09	08/31/10	5,000 *
Pfizer Inc.	Bovine Genome	04/01/09	03/31/10	5,000 *
Research and Diagnostic Systems, Inc.	Gene Expression and Signaling in the Immune System	10/01/09	09/30/10	2,500 *
Roche Diagnostics Corporation	Personal Genomes	03/01/09	02/28/10	35,000 *
	Bovine Genome	03/01/09	02/28/10	10,000 *
	The Biology of Genomes	03/01/09	02/28/10	35,000 *
University of Illinois	Molecular and Cellular Biology of Plasminogen Activation	05/01/09	04/30/10	2,500 *
University of Missouri	Bovine Genome	04/01/09	03/31/10	9,600 *
University of Pennsylvania	Single-cell Techniques	04/01/09	03/31/10	5,000 *
Dr. Charles Weissmann	2010 CSHL Symposium Premeeting to Celebrate the 75th Symposium	12/01/09	11/30/10	10,000 *
The Wellcome Trust	History of the Human Genome Project	04/01/09	03/31/10	14,500 *
Carl Zeiss MicroImaging, Inc.	Single-cell Techniques	04/01/09	03/31/10	3,500 *
Library Support				
James R. Hudson, Jr.		09/16/09	09/15/10	75,000 *
Ellen Brenner Memorial Fund		12/22/09	12/21/10	2,000

[1]Includes direct and indirect costs
*New grants awarded in 2009

DOLAN DNA LEARNING CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2009 Funding*[†]
FEDERAL GRANTS			
National Institutes of Health	Science Education Partnership Award (SEPA): Nationwide Dissemination of *Inside Cancer* Internet Site	08/07–07/10	$ 97,358
National Science Foundation	Course, Curriculum, and Laboratory Instruction (CCLI) Program: Nationwide Dissemination of RNAi Curriculum	9/07–8/10	165,361
National Science Foundation, American Association for the Advancement of Science	National Science Digital Library: Meta-tagging DNALC Internet Content for BiosciEdNet	10/05–9/09	65,240
National Science Foundation, University of Arizona	Educational Outreach for *iPlant:* A Cyberinfrastructure for Plant Sciences	2/08–1/13	409,411
National Science Foundation, Cornell University	*Weed to Wonder* Internet Site Development: Educational Outreach for Functional Genomics of the Maize Shoot Apical Meristem	9/08–8/10	190,156
NONFEDERAL GRANTS			
Bank of America	Scholarships for Minority and Underserved Students on Long Island	2009	25,000
Dana Foundation	*Genes to Cognition (G2C) Online* Internet Site Development	10/04–3/09	$14,725
Dana Foundation	*Harlem DNA Lab* Operating Support	3/09–2/12	118,298
Dialog Gentechnik	DNALC Licensing	2009	62,400
Hewlett Foundation	*Genes to Cognition (G2C) Online* Internet Site Dissemination and Evaluation	10/05–4/10	107,387
HHMI Foundation	Pre-College Science Education Initiative: NYC Teacher Professional Development	9/07–8/12	136,126
Lounsbery Foundation	Biotechnology Footlocker Program: *Harlem DNA Lab*	11/09–8/10	51,916
National Grid Foundation	Scholarships for Minority and Underserved Students in the Brentwood Union Free School District	5/09–5/10	10,000
North Shore-LIJ Health System	DNALC *West* Operating Support	2009	50,000
Porter Foundation	Scholarships for Minority and Underserved Students at *Harlem DNA Lab*	3/08–3/10	31,850

The following schools each contributed $1000 or more for participation in the *Curriculum Study* Program:

Bethpage Union Free School District	$ 1,500	Manhasset Union Free School District	$ 2,500
East Meadow Union Free School District	3,000	Massapequa Union Free School District	3,000
Elwood Union Free School District	3,000	Northport–East Northport	2,500
Garden City Union Free School District	1,500	North Shore Central School District	3,000
Great Neck Union Free School District	3,000	North Shore Hebrew Academy	3,000
Green Vale School	3,000	Oceanside Union Free School District	3,000
Half Hollow Hills Central School District	1,500	Oyster Bay–East Norwich School District	1,500
Harborfields Central School District	1,500	Plainedge Union Free School District	3,000
Herricks Union Free School District	1,500	Plainview–Old Bethpage Central School District	3,000
Huntington Union Free School District	3,000	Port Washington Union Free School District	1,500
Island Trees Union Free School District	1,500	Ramaz School	1,500
Jericho Union Free School District	1,500	Roslyn Union Free School District	3,000
Kings Park Central School District	1,500	Sachem Central School District	3,000
Lawrence Union Free School District	3,000	South Huntington Union Free School District	1,500
Levittown UFSD	3,000	Syosset Central School District	1,500
Locust Valley Central School District	1,500	West Hempstead Union Free School District	3,000
Long Beach City School District	3,000	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:

Amityville Union Free School District	$ 1,200	Locust Valley Central School District	$ 7,750
Bay Shore Union Free School District	6,000	Merrick Union Free School District	2,250
Bellmore Union Free School District	4,000	Mott Hall V, NYC	1,200
Bellmore–Merrick Central HS District	16,000	MS 181, NYC	1,000
Bethpage Union Free School District	2,250	MS 447 The Math and Science Exploratory School, NYC	2,480
Commack Union Free School District	5600	North Bellmore Union Free School District	4,300
Deer Park Union Free School District	1,600	North Shore Central School District	1,500
Eastern Middle School, Greenwich, CT	2,900	North Shore Hebrew Academy	1,000
East Meadow Union Free School District	2,550	Oceanside Union Free School District	1,500
East Williston Union Free School District	5,600	Old Westbury School of the Holy Child	3,250
Elmont Union Free School District	1,800	Oyster Bay–East Norwich Central School District	2,475
Floral Park–Bellerose Union Free School District	6,500	Rockville Centre Union Free School District	2,400
Friends Academy, Locust Valley	2,350	Roslyn Union Free School District	3,600
Garden City Union Free School District	10,950	St. Dominic Elementary School, Oyster Bay	4,050
Great Neck Union Free School District	11,800	St. Edward the Confessor School, Syosset	2,025
Half Hollow Hills Central School District	7,625	Syosset Central School District	32,500
Hauppauge Union Free School District	3,000	Three Village Central School District	2,200
Herricks Union Free School District	2,700	Trinity Regional School	1,100
Huntington Union Free School District	4,350	Valley Stream 13 Union Free School District	1,200
Jericho Union Free School District	8,125	Yeshiva Darchei Torah	1,400
Lawrence Union Free School District	6,700	Yeshiva of North Jersey, NJ	2,100
Lindenhurst Union Free School District	1,400		

[†]Includes direct and indirect costs

BANBURY CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2009 Funding*
FEDERAL SUPPORT			
NIH–National Institute of Mental Health	The 1st Annual NIMH-Sponsored Brain Camp	2009	$ 37,177
NIH–National Institute on Drug Abuse	Neurobiology of Depression: From Molecules to Mood	2009	5,580
NIH–National Institute of Neurological Disorders and Stroke	From Infection to Neurometabolism: A Nexus for CFS	2009	35,000
NIH–National Institute of Mental Health (through a grant to University of Illinois)	New Developments in Fragile X Syndrome: From Basic Mechanisms to Therapeutics	2009	51,273
National Science Foundation	NSF Workshop: A Vision for Plant Biology	2009	915
NONFEDERAL SUPPORT			
Meeting Support			
AstraZeneca Pharmaceuticals	Neurobiology of Depression: From Molecules to Mood	2009	7,000
Biotechnology and Biological Sciences Research Council (BBSRC)	NSF Workshop: A Vision for Plant Biology	2009	2,745
Boehringer Ingelheim Fonds	Science: Get it Across!	2009	67,991
CFIDS Association of America	From Infection to Neurometabolism: A Nexus for CFS	2009	15,753
Cold Spring Harbor–Pioneer Collaborative Research Program	Epigenetic Inheritance, Gene Regulation, and Plant Development	2009	47,049
Deutsche Forschungsgemeinschaft (DFG)	NSF Workshop: A Vision for Plant Biology	2009	915
The Gordon and Betty Moore Foundation	Aquatic Plants: Environment, Energy, and Evolution	2009	50,000
Hoffmann-LaRoche, Inc.	Neurobiology of Depression: From Molecules to Mood	2009	10,000
Hope for Depression Research Foundation	Neurobiology of Depression: From Molecules to Mood	2009	4,593
Johnson & Johnson	Neurobiology of Depression: From Molecules to Mood	2009	10,000
Lilly Research Laboratories	Neurobiology of Depression: From Molecules to Mood	2009	6,600
NARSAD, The Brain and Behavior Research Fund	Promoting Research on Severe Mental llness	2009	15,000
OSI Pharmaceuticals, Inc.	Feedback Networks in the Intersection of Metabolism and Receptor Tyrosine Kinase Signaling	2009	32,132
The Salk Institute	NSF Workshop: A Vision for Plant Biology	2009	8,235
Simons Foundation	Promoting Research on Severe Mental Illness	2009	5,000
The Stanley Research Foundation	Psychiatric Genetics: Current Progress and Future Directions	2009	17,823
The Swartz Foundation	Searching for Principles Underlying Memory in Biological Systems	2009	44,355
Time for Lyme, Inc.	Coinfections in Lyme Disease	2009	5,070
University of Arizona	iPlant Collaborative	2009	23,078
The Wellcome Trust	International Catalog for the History of the Human Genome Project	2009	12,369
World Heritage Foundation–Prechter Family Fund	Promoting Research on Severe Mental Illness	2009	5,000
Wyeth Pharmaceuticals	Neurobiology of Depression: From Molecules to Mood	2009	5,000

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 2009 as the economic difficulties began to take effect. The year 2010 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 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, and this is mailed to approximately 30,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory website on the Meetings Office and Banbury Center pages. Members in 2009 were

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Life Technologies (Applied Biosystems & Invitrogen)
New England BioLabs, Inc.
OSI Pharmaceuticals, Inc.
Sanofi-Aventis
Schering-Plough Research Institute

DEVELOPMENT

June 12, 2009 was an important day in the history of Cold Spring Harbor Laboratory as we dedicated six new buildings to expand our research programs in cancer, autism, and schizophrenia, and to house a new program in quantitative biology. At the dedication were many of our long-time friends who participated in the $200 million capital campaign and helped to make the blueprints from 2000 into such a beautiful reality. One of the highlights from the ceremony for me was the presence of 17 members of the Quick family. Jean and Les Quick, whose names grace one of the new buildings for cancer research, would be so proud to know that their legacy of philanthropy and family continues. Brothers Tom and Peter Quick both served on the Laboratory's campaign cabinet and provided much help and support.

Our summer jubilation was dampened later in the fall with the passing of Don Axinn, one of the staunchest advocates for this building project. Not only did Don name the last building in the complex, but he also lent his real-estate development expertise chairing the Laboratory's building committee. His advice and attention to our facilities team were quite remarkable and unprecedented. Don's boundless energy was thwarted in the last months by his esophageal cancer which metastasized, but he left this world knowing that these buildings will be home to the breakthrough work of young scientists like Dr. Mikala Egebald who has developed a technology to determine why and how tumors metastasize. Don taught me many life lessons that I am just beginning to appreciate, and all of us here at the Laboratory will miss him terribly.

As 2009 draws to a conclusion, I would like to thank all of you who care and support the growth of Cold Spring Harbor Laboratory as we approach this new decade of discovery.

Charles V. Prizzi, *Vice President for Development*

The Quick family at the Hillside Campus dedication ceremony

President's Council

Members of the President's Council contribute at least $25,000 annually to support CSHL Fellows, unusually talented young scientists who embark on independent research directly after graduate school. Each year, members gather for a spring reception and a fall retreat to explore a chosen topic of broad public interest that relates to CSHL research.

The April reception hosted by CSHL Chairman Eduardo Mestre and his wife Gillian featured a presentation by 23andMe by cofounders Linda Avey and Anne Wojcicki (wife of Google cofounder Sergey Brin). Companies such as 23andMe encourage individuals to access and learn about their own genetic information, and they compile data to create common, standardized resources of genetic profiles with the hope of accelerating personalized medicine. The October retreat included talks about personal genomes related to health, privacy, the criminal justice system, human origins, and advancements in genome sequencing technology.

President

Dr. Bruce Stillman

Chairpersons

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Mr. George F. Ohrstrom
Dr. and Mrs. James M. Stone

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23andme founders (*left to right*) Linda Avey and Anne Wojcicki with Connie de Balmann, CSHL Trustee Kristina Davison and Silda Wall Spitzer at the 2009 Spring President's Council Reception.

2009 Fall President's Council attendees listen as a Cold Spring Harbor Laboratory scientist Doreen Ware discusses her research.

Cold Spring Harbor Laboratory Association (CSHLA)

Once again, the CSHL Association annual fund goal was exceeded, and we raised $1,153,616. We were proud to have CSHL alumni and new President of the Howard Hughes Medical Institute Bob Tjian give a lively overview of CSHL's impact on the world of biology at our annual meeting in January. For the first time in years, we took a break from our annual spring benefit, but supporters responded generously to our "noninvitation" and donated more than $80,000. The annual Symposium this year was on Evolution, and neighbors and friends at nearly 20 different dinner parties especially enjoyed meeting paleontologists, anthropologists, and philosophers along with geneticists and molecular biologists from around the world. Fife and Heather Whitman warmly welcomed CSHL's major donors to their meticulously restored historic Jones family home, Hill House, on a memorably balmy Sunday evening in early October. We are grateful to Laboratory Association President Tim Broadbent for his energetic leadership of our board and look forward to another eventful year of friend raising and fund raising in 2010.

Directors

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2009 CSHLA Hike

Dolan DNA Learning Center Advisory Boards

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 liaison to the Long Island and New York City 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 2009, more than $200,000 was raised through these events.

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CSHL President Bruce Stillman (*left*) with CSHL Trustee Marilyn Simons and Jim Simons at the 2009 Double Helix Medals Dinner

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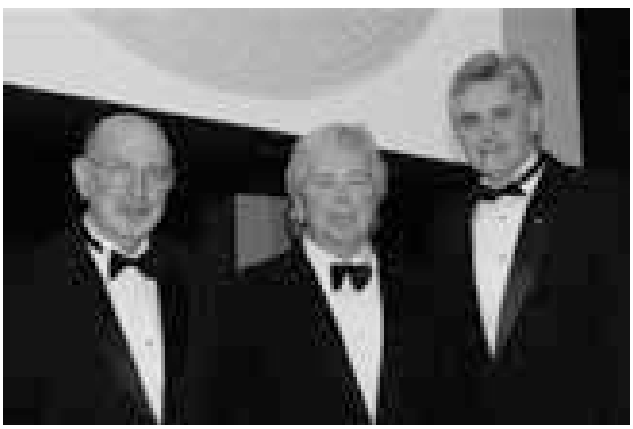

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Michael J. Fox with CSHL President Bruce Stillman during a visit to the Laboratory

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Valery Krizhanovsky*
Jayant Kulkarni
Duda Kvitsiani
Tali Lavy*
Seung-Jae Lee*
Young Koung Lee
Darrin Lewis
Li Li
Ying Lin
Yilei Liu
Mukesh Lodha
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Louis Manganas
Yuntao Mao
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Shane Edward McCarthy
Christoph Miething
Sylvain Mitelheiser
Nael Nadif Kasri
Shuang Ni
Dawid Nowak
Michaela Oswald-Pisarski
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Soonju Park
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Anirban Paul
Shilpi Paul
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Thomas Schalch
Andrea Schorn
Ulla Schwertassek
Damianos Skopelitis
FNU Sneh Lata
Naoki Takada
Chunlao Tang
Man-Hung Eric Tang
Hiroki Taniguchi
Milos Tanurdzic
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Ante Tocilj
Vladimir Vacic
Vasily Vagin
Daniel Valente
Emma Vernersson Lindahl
Chia-Lin Wang
Haibin Wang
Zihua Wang
Michael Wittinger
Kazimierz Wrzeszczynski
Caizhi Wu
Qiaojie Xiong
Xianfeng Xu
Jianchao Yao
Zhan Yao
Zhiguang Yuchi
Miguel Zaratiegui Biurrun
Lixing Zhan
Bin Zhang
Zhihua Zhang

Graduate Students

Timour Baslan
Philippe Batut
Mitchell Bekritsky
Megan Bodnar
Dario Bressan
Ralph Burgess
Ying Cai
Joseph Calarco
Matthew Camiolo
Stephane Castel
Jiook Cha
Sudipto Chakrabortty
An-Yun Chang
Deblina Chatterjee
Fauzia Chaudhary
Sihem Cheloufi
Hyejin Cho
Sungwook Choi
Carrie Clendaniel
Galen Collins
Michael Cressy
Noelle Cutter
Benjamin Czech
Shipra Das
Kristen Delevich
Ashesh Dhawale
Saya Ebbesen
Melanie Eckersley-Maslin
Elad Elkayam
Yaniv Erlich
Christof Fellmann
Xin Feng
Silvia Fenoglio
Patrick Finigan
Oliver Fregoso
Yu Fu
Wee Siong Goh
Eyal Gruntman
Paloma Guzzardo Tamargo
Jong Jin Han
Aftabul Haque
Miao He
Kyle Honegger
Ikuko Hotta
Jiahao Huang
Dong-Woo Hwang
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Sang-Geol Koh
Marek Kudla
Canan Kuscu
Mary Kusenda
Matthew Lazarus
Amy Leung
Wangzhi Li
Wanhe Li
Willey Liao
Katie Liberatore
Guang Lin
Haixin Liu
Zhijie Liu
Elena Lum
Hiroshi Makino
Colin Malone
Katherine McJunkin
Ozlem Mert
Yifan Mo
Adam Mohamed Naguib
Antoine Molaro
Elizabeth Nakasone
Nicholas Navin
Diana Orozco
Hassana Oyibo
Shraddha Pai
Michael Pautler
Ian Peikon
Zinaida Perova
Maria Pineda
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Mathangi Ramesh
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Suyash Shringarpure
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Elvin Wagenblast
Lei Wang
Zhenxun Wang
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Ming Yang
Yang Yang
Yu-Ting Yang
Jia-Ray Yu
Cinthya Zepeda Mendoza
Jianping Zhang
Rui Zhao
Zhen Zhao
Xin Zhou
Petr Znamenskiy

Scientific Informatics

Peter Andrews
Abhishek Bhandari
Jer-Ming Chia
Aaron Chuah
Alexander Dobin
Assaf Gordon
Sonali Jha

*Employee of the Howard Hughes Medical Institute

Jude Kendall
Sohail Khan
Melissa Kramer
Sunita Kumari
Anthony Leotta
Wei Lin
Zhenyuan Lu
Weijun Luo
Vladimir Makarov
Andrew Olson
Martin Paradesi
Shiran Pasternak
Kandasamy Ravi
Liya Ren
Joshua Stein
James Thomason
Liya Wang
Xuehong Wei
Chenghai Xue
Seungtai Yoon
Charles Youens-Clark

Research Associates

Carmelita Bautista
Barry Burbach
Hilary Cox
Jorg Drenkow
Cexiong Fu
Zsolt Lazar
Lisa Manche
Linda Rodgers
Patricia Wendel
Xiaoqun Zhang

Laboratory Facility Managers

Sabrina Boettcher*
Nancy Dawkins-Pisani
Stephanie Goldsmith*
Stephen Hearn
Christopher Johns
Pamela Moody
Timothy Mulligan
Joseph Simorowski
Spencer Teplin
Jennifer Troge
Ming Wang

Laboratory Manager

Martha Daddario

Scientific Administrators

Paloma Anderson
Sadie Arana
Christine Bedell
Patricia Bird
Lynn Boss*
Maria Chapelle
Becky Dong
Irene Gill
Carol Gundersen
Jo Leonardo*
Idee Mallardi
Barbara Matusik
Sandra Michelsen
Stephanie Peragallo
Madeline Wisnewski

Special Projects Technician

Lori Spiegel

Laboratory Technicians

Sai Dipikaa Akshinthala
Chandni Ashar
Yanghuan Bao
Kimberly Bell
Jacqueline Cappellani
Laura Cardone
Thomas Caulfield
Yon Chang
Xin Cheng
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Tara Spencer
Maya Strahl
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Kevin Tranchina
Amy Valentine
Eric Wang
Huaien Wang
Jessica White
Scilla Wu
YoungJoo Yang
Tara Zadrozny
Martha Zeeman
Lifang Zhang

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Christopher Algieri
June Blanchford
Patricia Brady
Florinda Chaves
Terrance Chisum
Maria Hallaran
Qiaoling He
Salvador Henriquez
Adriana Hincapie
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Research Administration

Maureen Bell

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CSHL Press

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Leah Steuer
Rena Steuer
Sharon Story
Hillary Sussman
Linda Sussman
Christopher Vacca
Denise Weiss

*Employee of the Howard Hughes Medical Institute

Dolan DNA Learning Center

David A. Micklos, *Executive Director*
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Elna Carrasco
John Connolly
Lauren Correri
Jennifer Galasso
Cornel Ghiban
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Eun-Sook Jeong
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Watson School of Biological Sciences

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Rita Wienclaw

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Peter DiLauro
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Frank Falco
Kelvin Flores
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David Trimboli
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Kimberly Bronson
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Bruce Fahlbusch
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James Duffy
Catherine Eberstark
Philip Renna
Peter Tarr

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Diane Errico
Donna Gavin
Jill Hemish
Joanne Janelli
Ruta Ledins
Stacy Leotta
Kathleen Wall-Rooney

Technology Transfer

John P. Maroney, *Vice President and In-House Counsel*
Elizabeth Cherian-Samuel
Vladimir Drozdoff
Lorraine McInerny
Limin Wen

RESEARCH STAFF DEPARTURES DURING 2009

Assistant Professor

Jacek Skowronski

Adjunct Professors

Peter D'Eustachio
Timothy Tully

Adjunct Associate Professor

Dmitri Chklovskii

Research Assistant Professor

Zhenyu Xuan

Visiting Scientists/ Collaborative Scientists

Aniruddha Das
Carolina Elias
Hao Lin
Zefeng Zhang

Senior Fellow

Alexey Aravin

Clinical Fellow

Timothy Pardee

Postdoctoral Fellows

Victoria Aranda Calleja
Yoselin Benitez Alfonso
Anka Bric
Santanu Chakraborty
Samit Chatterjee
Shuaili Chen
Wen Yu Chung

Anne Daulny
Ebru Demir
Katalin Fejes Toth
Gidon Felsen
Fuqiang Geng
Vladimir Grubor
Nobuhiko Hiramatsu
Katarzyna Hrecka
Min Hsuan Chen
Bing Hu
Robyn Johnston
William Keyes
Sandra Kuhlman
Claus-Dieter Kuhn
John Kurland
Yang Liu Sullivan
Jernej Murn
Gonzalo Hugo Otazu Aldana
Ananda Sarkar
Richard Slotkin
Vihra Sotirova
Clinton Whipple
Zuo Zhang

Scientific Informatics

Jason Bohland
Hemant Bokil
Gopalakrishnan Gopinathrao
Alexander Kanapin
Chengzhi Liang
Steven Marks
Marcela Monaco
Timothy O'Connor
Deepa Pai
Andrew Sundstrom
Guanming Wu
Wei Zhao

Research Associate

Simone Salghetti